Biological Indicators of Stream Condition in Montana Using Benthic Macroinvertebrates

Prepared for:

Montana Department of Environmental Quality

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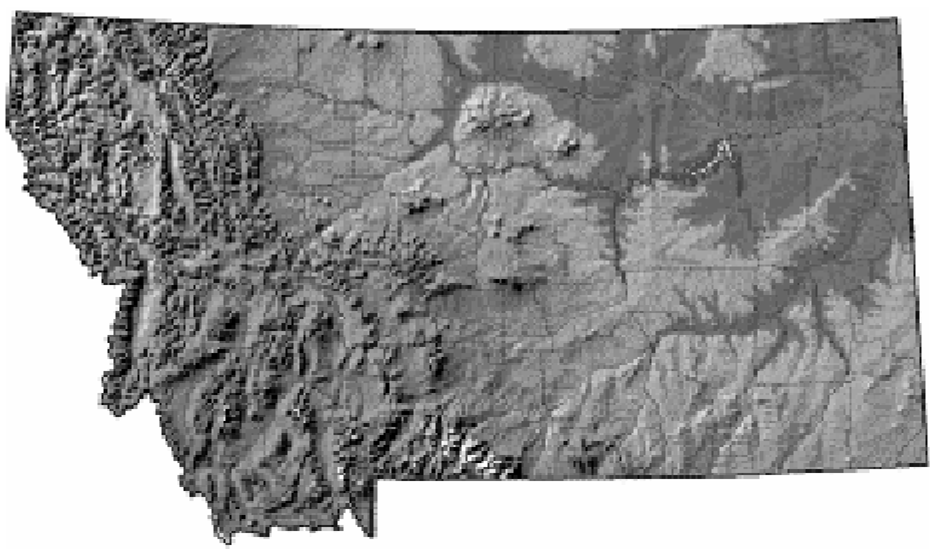

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ABSTRACT

The Montana Department of Environmental Quality (MDEQ) uses biological condition as the primary indicator of ecological quality of streams and watersheds. Historically, they used three multimetric indexes (MMI) patterned on the concept of the Index of Biological Integrity for different areas of the state, one each for 1) Mountains, 2) Foothills and Valleys, and 3) Plains. The purpose of this project was to recalibrate the benthic MMI using a larger, more recent database and to develop a second type of biological indicator, a predictive model based on the River Invertebrate Prediction and Classification System (RIVPACS), known as the observed/expected (O/E) model. Compiling benthic macroinvertebrate data collected from >950 Montana wadeable stream sites by multiple programs and agencies, a unified database was constructed, and prepared for analysis. Reference and stressor site criteria were developed and applied to the overall dataset, resulting in 133 reference and 71 stressor sites. The overall taxonomic list (all sites and all taxa) was evaluated to establish an operational taxonomic unit (OTU) for each taxon, defining the hierarchical level at which each would be considered distinct and unambiguous. Site classes were determined for the multimetric index model using multivariate analysis (nonmetric multidimensional scaling [NMS]) of Bray-Curtis similarity indexes (BC). The resulting classification (Mountains, Low Valleys, and Plains) paralleled the prior site classification used by Montana. Metrics were tested for range, capacity for detecting the presence of stressor conditions (accuracy, calculated as discrimination efficiency [DE]), and redundancy. There were seven metrics selected for the Mountains class, and combined as an MMI, had a DE of 100%; the 5-metric MMI of the Low Valleys had a DE of 94%; that for the Plains (also of five metrics) was 77.4%. The 90% confidence interval for all three indexes ranges from 6.9 to 9.6 points on a 100 point scale. Verification using a separate dataset was favorable for the Mountains and Plains; the total number of sites for the Low Valleys was too small to allow for a verification test. For the RIVPACS model, the cluster analysis algorithm unweighted pair-group method with arithmetic mean (UPGMA) was used with BC as input, resulting in 5 groups of streams. Evaluating up to 15 different predictor variables using all subsets software, the final model used 5 predictor variables: latitude, longitude, mean maximum annual air temperature, located in Columbia River basin (y/n), and log watershed area. The mean O/E value of the calibration sites was 0.99 (s. dev. = 0.17), substantially better than that associated with the null model; the model also accounted for approximately 88% of the taxonomic variability among samples. Both indicators will be implemented as tools for interpretation of benthic macroinvertebrate field samples, and ultimate assessment of streams as impaired or nonimpaired.

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1.0 Introduction

1.1 Assessment Needs

EPA advocates the use of biological criteria and development of numeric biocriteria to assist states in decision-making and management for 305(b) reporting, 303(d) lists, TMDL development, and watershed restoration (NRC 2000). Biological assessments provide a direct measure of the status and functioning of an aquatic community of plants and animals as well as biological benchmarks for water quality management programs. Bioassessments and biocriteria can be used to list impaired water bodies on the 303(d) lists, to de-list water bodies that have healthy aquatic communities and to assess the effectiveness of TMDL control measures to restore aquatic life uses in water bodies. Given the application of biological data in the 303(d) and TMDL process, the State's bioassessment methods are in need of critical evaluation and improvement.

Among the needed improvements to the DEQ bioassessment program is the development of biological assessment techniques that are calibrated using multiple statewide datasets that are now available, as well as a complete re-designation of reference sites. Such a recalibration will enhance the State's ability to make impairment determinations that are applicable over the entire state and using samples collected by various agencies or sampling protocols. It will also strengthen bioassessment results through the use of scientifically defensible model development procedures and analyses. The purpose of this study is to improve the bioassessment capabilities of Montana Department of Environmental Quality (DEQ) by recommending indexes and models resulting from analysis of the most current and complete macroinvertebrate and site characteristic data collected throughout the state.

This report documents the development of two biological indexes, one a multimetric index, and one a multivariate predictive index, for use in the assessment of Montana streams and to support the 305(b) report and 303(d) list of impaired waterbodies. The specific questions investigated in this study were:

- What is the most appropriate site classification for assessing stream conditions across the diverse landscape and physiographic regions of Montana?
- Which metrics are most appropriate for use in a Montana multimetric macroinvertebrate stream condition index?
- What predictive model is best for Montana streams?
- What biological index thresholds indicate the degree of comparability of Montana streams to reference condition?
- What programmatic changes can be made to better assess stream conditions throughout Montana in the future?

1.2 Benthic Macroinvertebrates

Benthic macroinvertebrates have long been recognized as a valuable assemblage for indicating biological conditions in streams. DEQ has collected biological data, (periphyton and macroinvertebrates), for more than twenty years. The aquatic entomology experts of Montana

DEQ originally developed macroinvertebrate multimetric indices for regions of the state. Historically, DEQ has used three multimetric indices for assessing aquatic life use attainment across ecoregions of the state – one each for 1) Mountains, 2) Foothills and Valleys and 3) Plains (Bollman 1998). The Foothill and Valley index was revised from its original format based on discriminant analysis conducted by Rhithron Biological Associates. Other indices have been developed that have applicability in Montana. They include an index for application in Prairie streams by Bramblett et al. (2003), indices calibrated for three distinct ecological regions of Montana by Marshall and Kerans (2003), and an index for the basin and plains areas of Wyoming by Stribling et al. (2000).

1.3 Tools for Biological Assessment

Two analytical approaches were taken to assess biological condition in Montana streams. The two approaches – multimetric indices and predictive models – are similar in that they attempt to discern biological differences between those sites that have minimal landscape pressures (reference) from sites with increasing degrees of pressure (non-reference or degraded). The two approaches differ in the way sites are classified into similar natural groupings and in the way the biological information is summarized.

In the **multimetric approach**, sites are classified into *distinct* natural groups based on biological similarities that can be explained by environmental variables (Barbour et al. 1999, Gerritsen et al. 2000). Identification of those environmental variables that determine class membership is based on *a priori* investigation of biological similarity in relation to environmental variables that may have a natural effect on reference community composition. If such a clear division of reference biological site types exists, each site is assigned to one of the multiple classes and index development proceeds.

Metrics comprising a multimetric index (MMI) are characteristics of the benthic macroinvertebrate assemblage that change in some predictable way with increased human influence that alters environmental conditions (Barbour et al. 1996). The metrics are based on taxonomic diversity and composition, stressor pollution tolerance, feeding mechanisms, habit (mode of attachment or locomotion), and voltinism (reproductive periodicity). Beginning with a suite of metrics, each is evaluated in terms of responsiveness to stressors, along a categorical gradient of stressed landscape condition (reference vs. degraded). The multimetric index is a mathematical combination of multiple metrics that measures the overall response of the community to environmental alteration and stressor conditions (Karr et al. 1986, Barbour et al. 1995). Such a measure of the structure and function of the biota (using a regionally-calibrated multimetric index) is an appropriate indicator of ecological quality, reflecting biological responses to changes in physical habitat quality, the integrity of soil and water chemistry, geophysical process, and land use changes (to the degree that they affect the sampled habitat and water quality).

Multimetric, invertebrate indexes of biotic integrity (IBI), also variously called ICI (Invertebrate Condition Index; Ohio EPA 1989), B-IBI (Benthic IBI; Kerans and Karr 1994), and SCI (Stream Condition Index; Barbour et al. 1996; Burton and Gerritsen 2003), have been developed for many regions of North America and are generally accepted for biological assessment of aquatic resource quality (e.g., Gibson et al. 1996, Plafkin et al. 1989; Barbour et al. 1999, Southerland and Stribling 1995, Karr 1991). The framework for bioassessment consists of characterizing reference conditions upon which comparisons can be made and identifying appropriate biological attributes with which to measure the condition. Reference conditions are typically the "best available" conditions where biological communities are the closest to natural for the particular region or area. These reference conditions are taken to be representative of healthy ecosystems.

The **predictive modeling approach** allows assessment of biological condition or quality by estimating the taxonomic completeness of a standard sample (Hawkins 2006 [*in press*]). Taxonomic completeness is a fundamental aspect of biological integrity and is defined here as the proportion of the taxa that should occur in a sample that were actually sampled. The accuracy and precision of predictive modeling assessments depend on the quality of the model used to predict the taxa expected to occur in a sample collected from an individual site. These models describe how probabilities of capture of all taxa vary across naturally occurring environmental gradients, information from which the taxa expected at individual sites can be derived. In contrast to multimetric indexes, the performance of these models does not depend on calibration against presumed stressed sites. Models are calibrated only with reference site data. If models accurately predict the assemblage that should occur at a site under reference conditions, any deviation from these predictions is a direct measure of biological impairment.

The model is built such that the taxa occurring in the reference sites are used to predict taxa that are expected to occur in sites with similar environmental characteristics. Sites that are environmentally similar to a reference group are expected to have the taxa that occur in that group to the same degree as their environmental similarity, defined by the probability of class membership. The prediction of expected taxa and observation of those taxa actually occurring in the sample allows calculation of the degree to which a site is attaining its potential in biological diversity. This calculation is observed taxa (O) over expected taxa (E). Values of the ratio, O/E, theoretically can range from 0 to 1, with values of 1 implying reference conditions and values less than 1 implying biological impairment.

2.0 Data Sources and Organization

A robust dataset is the basis for developing any assessment tool. EPA is currently working with DEQ's data management section to migrate twenty years of biological data into STORET, the national storage and retrieval warehouse for monitoring data. As of this project, DEQ biological data were entered for samples collected between 1990 and 2003. These data were collected for three state programs - Reassessment Monitoring, the Fixed Station Network, and Reference Sites. Together, they comprised the bulk of the analytical data set (Table 1). Other data were available from a recent nutrient assessment program (Suplee 2004) and comparability studies that addressed differences in mesh sizes and sampling protocols (Jessup et al. 2005).

Macroinvertebrate data have been collected throughout Montana by agencies other than DEQ using similar, but not identical, sample collection protocols. Because analyses and others are more robust with a larger sample size and have a more complete geographic coverage, data from several of these agencies were included in the analysis (Table 1). These additional data sets included Western EMAP and Eastern Montana Regional EMAP (US EPA Environmental

Monitoring and Assessment Program), USU-STAR (Utah State University Western Center for Monitoring and Assessment of Freshwater Ecosystems, Science to Achieve Results program), U.S. Forest Service Reference Sites, and the Natural Heritage Program Reference Sites. All the programs sampled a similar size area using comparable kick methods. The principal difference among the programs was the target subsample size, which ranged from 300 specimens to the whole sample.

Table 1. Data sets compiled for use in developing indices and models (with numbers of stations containing biological data).

Montana DEQ (1990-2003) (590)	**EMAP Western Pilot (Montana stations) (88)**
Reassessment Monitoring	REMAP (Eastern Montana) (76)
Fixed Station Network	U.S. Forest Service Reference Sites (75)
Reference Sites	Natural Heritage Program Reference Sites (52)
Nutrient Pilot Study (22)	Science to Achieve Results (STAR) (35)
Mesh Comparability Study (15)	
Protocol Comparability Study (10)	

A comparability study that investigated sample biases due to net mesh size and collection protocol revealed that samples collected using the various mesh sizes and four protocols were comparable in the context of site assessment (Jessup et al. 2005). The protocols that were assessed and were found to be similar included DEQ’s traveling kick, EMAP reachwide, EMAP targeted riffle, and Surber samples. The biggest differences in metric results from different protocols were associated with taxa richness metrics based on different subsample target sizes. These differences were rectified through rarefaction (computational re-sampling) of those metrics that were counts of taxa. Most of the data compiled for this study were collected using protocols similar to those evaluated. Few samples that were included in the analysis were collected using unevaluated protocols (Hess or jab). For these protocols, we assumed favorable comparability.

Data were compiled in a customized Microsoft Access database, the Ecological Data Application System (EDAS). The database was capable of storing data by agency, protocol, station, sample, and taxon or variable. This dataset included benthic macroinvertebrate, physical characteristics (landscape statistics derived from a Geographic Information System), and water chemistry data. Through queries with taxa attribute tables, manipulations were possible to retrieve taxa lists, sample metrics, and environmental data that were necessary in subsequent analytical steps.

3.0 General Data Preparation

3.1 Reference Conditions

Most biological assessment models evaluate the biological condition of a waterbody relative to some expected or reference condition. The biological communities of relatively undisturbed “reference” streams are representative of healthy ecological communities expected to occur under the natural range of relatively undisturbed habitat, climate, geomorphology, and other physico-chemical characteristics of a region. A simple metaphor would be the use of 98.6 degrees as a "reference" for human body temperature. That target represents an "average" for relatively healthy individuals. It was likely derived by defining a population of relatively "healthy" individuals using a set of criteria to define an expected healthy condition and then averaging the temperatures of all of those meeting the criteria.

In addition to the use of reference sites, the multimetric approach required the identification of stressed sites. In this approach, indexes are constructed based on stream biological community characteristics that best discriminate between reference and stressed streams. As a result, it was necessary to develop both reference and stressed site criteria to identify sites for building these models.

The effort to identify reference sites was completed by the DEQ Water Quality Standards section (Suplee et al. 2005). In brief, their process included seven screening steps, each of which considered a different aspect of the physical and chemical characteristics of the site. For a site to be designated reference, criteria from all seven steps must be passed. The screening criteria included professional judgments, data completeness, and variables including road density, timber harvest intensity, agricultural land use, metals standards violations, and mining intensity. Sites from the Nutrient Pilot Study and STAR project (Suplee 2004, Stoddard et al. 2005) did not go through the DEQ evaluation process. These sites had already been extensively reviewed and were considered as established reference sites.

Identifying Degraded Sites

Identification of degraded (or, stressor) sites followed a similar screening and confirmation process. Within DEQ stations, potential degraded sites were identified as those on the 303(d) impaired waters list and those that failed EPA hardness adjusted heavy metals criteria. In consideration of the potential degraded sites and all others, degraded sites were identified based on the professional judgment of field biologists with local knowledge of the sampled streams. In addition, lists of degraded sites were solicited from EPA personnel familiar with EMAP and REMAP sampling stations.

Through the process described above, 133 reference sites and 71 degraded sites were identified (Appendix A). The multimetric and predictive modeling approaches had independent selection criteria that reduced the numbers of stations and samples that were actually used in the analyses. For instance, in cases where multiple samples were collected from a single site over time, only one sample per site was used in the analyses. In addition, when sites were within one kilometer of each other, only one site was used. The procedures used to identify subsets of the data are described below under the individual approaches.

3.2 Taxonomic Resolution

Assessment tools that rely on considering the number of taxa in a particular sample (e.g., richness metrics or O/E scores) require consistent taxonomic assignments of individual organisms to taxonomic groups. Ideally, all taxonomists would always assign any individual invertebrate to the same taxon. However, the quality of samples and the expertise of taxonomists vary (Stribling et al. 2003). As a result, specimens may not be identified to the same taxonomic resolution across all samples, and single samples may contain specimens identified to different hierarchical taxonomic levels. For example, one sample may have organisms identified to Diptera, Chironomidae, and *Chironomus*. In this example, it is impossible to tell whether these organisms represent one, two, or three taxa. Assuming that higher level identifications (order Diptera; family Chironomidae) are unique taxa, when they are not, would result in an inflated richness estimate. Such ambiguities in taxonomy require correction by applying consistent operational taxonomic rules to all samples.

We use the term operational taxonomic units (OTU) to specify common levels of identification that are applied across all samples, regardless of sample origin and processing history. We based assignments on a survey of samples collected in Montana and other western states. Decisions regarding the level of taxonomic hierarchy assigned to each OTU were based on the number of individuals that had been identified to different levels of resolution across all samples. For example, if most samples had individuals identified to *Limnephilus* and only a few individuals were identified to the family Limnephilidae, then individuals identified to Limnephilidae would be dropped from analyses. This loss of a potentially different taxon is the cost of ensuring that a standard taxonomy is used for all samples. In general, to ensure that analyses were based on as much unique ecological information as possible, the lowest taxonomic resolution possible was used for OTU assignments (e.g., genus or species).

Both MMI and O/E development used a practically identical set of OTUs. A notable exception is that for the multimetric approach individuals with higher level identifications can be retained for some composition metrics while being discounted in richness metrics. The predictive model approach does not use the higher level identifications at all. In this data set, chironomid midges (Diptera: Chironomidae) were assigned to sub-family level OTUs, because samples processed for the Forest Service used this level of taxonomic resolution for midges. Use of finer level OTUs for midges would have resulted in exclusion of Forest Service samples and thus a smaller sample size from which to develop indicators. Therefore, midge counts were compressed to the sub-family level for all analyses, except where noted in details below.

4.0 Multimetric Index (MMI) Development

The premise of the multimetric index development process is that physical and chemical disturbances are reflected by measurable changes in the structure and function of the benthic macroinvertebrate community. The benthic macroinvertebrate data from reference sites can be used to define a biological reference condition that is distinct from the degraded condition. Meaningful biological signals of disturbance are summarized in a multimetric index that can be used to evaluate biological integrity in sites of unknown quality. The development of a multimetric IBI calibrated on the benthic macroinvertebrate and environmental data collected in Montana streams follows a series of steps, as follows:

1. Compile the data (as described above);
2. Define reference and degraded sites (as described above);
3. Define site classes by stratifying reference biological conditions;

4. Calculate biological metrics and determine the sensitivity of each metric;
5. Combine appropriate metrics into index alternatives;
6. Select the most appropriate index for application within the site classes, and;
7. Assess performance of the index.

4.1 Site Classification

Multimetric indices are based on reference biological conditions and comparisons to those conditions. The reference condition is expected to vary due to natural differences among reference sites. If the differences are consistently associated with variable natural characteristics, then identification of multiple reference classes, or strata, will allow definition of multiple expectations of natural reference conditions. This will increase the chances of identifying truly degraded sites and decrease the chances of erroneously assessing a site as biologically impaired when it is actually of a different natural type.

Identifying classes among Montana's reference sites requires identification of biological groupings or assemblage types, association of the biological groups with natural variables, and sufficient reference samples for development of a multimetric index after dividing the reference sites into multiple classes. Biological groups are explored using non-metric multidimensional scaling (NMS), a comparison of taxa within each sample and an arrangement of the samples so that similar samples plot closer together than dissimilar samples in multiple dimensions. Natural environmental variables can be associated with the biological groups through visual inspection of the ordination diagrams and correlations with the biologically defined axes of the NMS diagram. NMS is a robust method for detecting similarity and differences among ecological community samples (McCune and Mefford 1999).

A site-by-taxon matrix was compiled with relative abundance of each taxon in each site. Rare and ambiguous taxa are not useful in the NMS ordination, and were eliminated. Similarity among reference biological samples was determined using the Bray-Curtis similarity measure. The Bray-Curtis (BC) formula is sometimes written in shorthand as

$$BC = 1-2W/(A+B)$$

where W is the sum of shared abundances and A and B are the sums of abundances in individual samples. The ordination software (PC-Ord, McCune and Mefford 1999) calculates a site-by-site matrix of BC similarity from which the arrangement of samples in the ordination diagram is derived. Multiple dimensions are compressed into two or three dimensions that we can perceive.

Samples arranged by biological similarity in the ordination diagram show a clear separation between mountainous and non-mountainous ecoregions (Figure 1). Ecoregion designations use the dominant level 3 ecoregion (Woods et al. 1999) in the catchment of the site (which is not necessarily the ecoregion at the site). Samples from the eastern plains (ecoregions 42 and 43) overlapped with each other, but not with the samples from the western and mountainous regions. A separate ordination with sites within only the mountainous ecoregions showed that samples from the lower, hotter, and drier valleys of the Middle Rockies (ecoregion 17) were somewhat distinct from other mountainous sites (diagram not shown). This distinction reflects historical classifications that identified foothill and valley sites as a separate class. An ordination of only plains sites did not reveal any further classes.

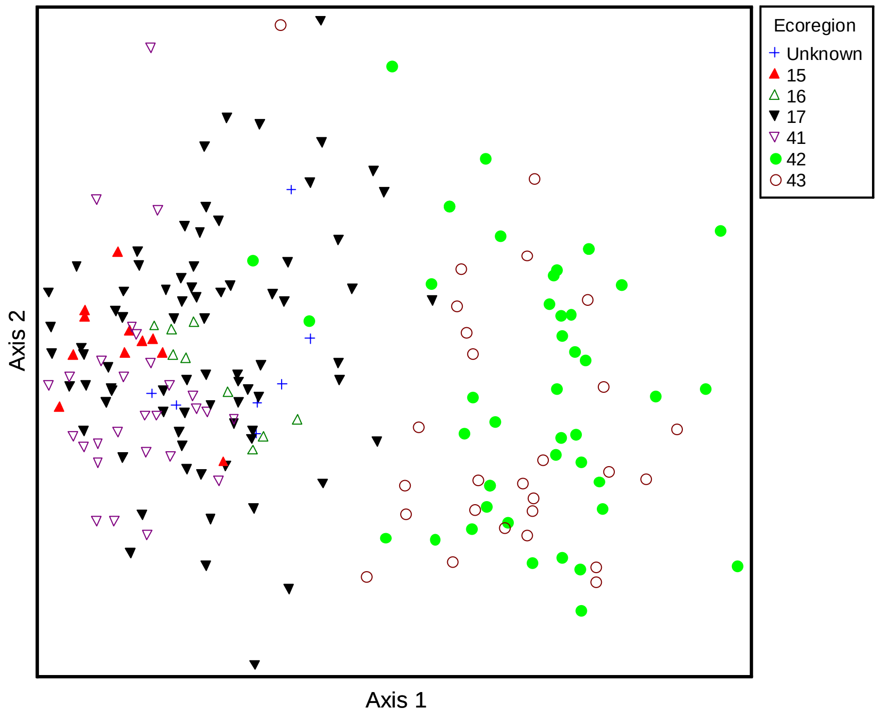

Figure 1. NMS ordination diagram of reference samples in taxa space, showing ecoregion designations. Ecoregion designations were not available for the sites represented by crosses.

Upon examination of metric distributions in reference sites (Appendix B), it appears that plains sites have different metric values compared to the other ecoregions. They have lower taxa counts and lower representation of sensitive individuals. They also have higher counts of non-insect taxa. In the mountains, the Middle and Canadian Rockies (ecoregions 17 and 41) have some metric distributions that appear slightly different than the Northern Rockies and Idaho Batholith (ecoregions 15 and 16), but not enough to warrant separate site classes. There are lower taxa counts in the Canadian Rockies, perhaps because these are cold, nutrient poor, and naturally harsh environments that do not support some taxa that are ubiquitous in other regions. Percentage metrics in the Canadian Rockies appear to be similar to the other mountainous ecoregions. The best classification scheme for Montana stream macroinvertebrates appears to have three site classes that parallel classes previously defined by DEQ (Figure 2, Table 2)

4.2 Metric Calculations and Responses to Stress

A biological metric is a numerical expression of a biological community attribute that responds to human disturbance in a predictable fashion. A suite of commonly applied, empirically proven, and theoretically responsive metrics was calculated for possible inclusion in a multimetric index. Metrics were considered for inclusion on the basis of discrimination efficiency, ecological meaningfulness, and sufficient range of values. They were organized into six categories: richness, composition, functional feeding group, habit (mode of locomotion), voltinism (reproductive periodicity), and pollution tolerance.

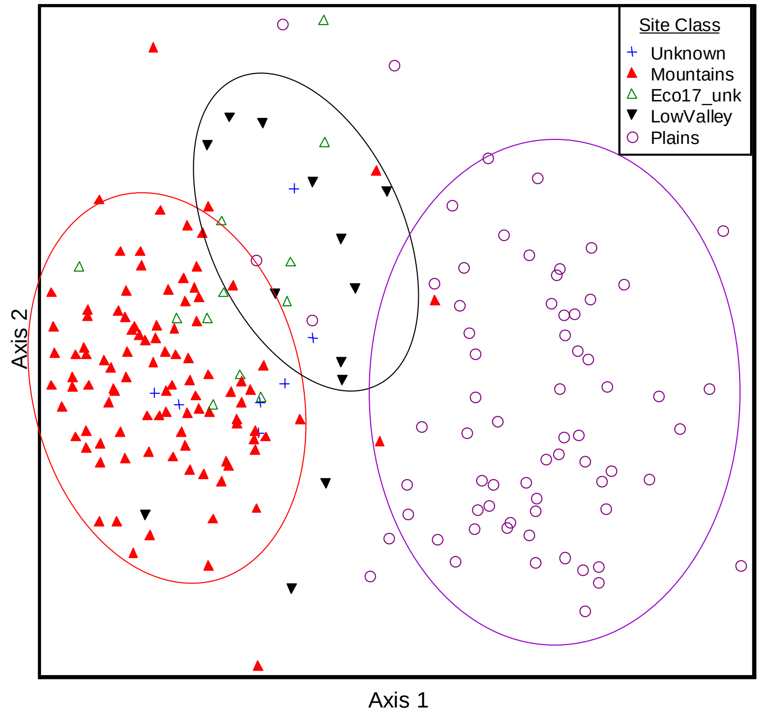

Figure 2. NMS ordination diagram of reference samples in taxa space, showing site class designations. Ovals enclose general groupings of sites. The open triangles are in ecoregion 17, but site characteristics for determining membership in the mountains or low valleys were not available. Ecoregion designations were not available for the sites represented by crosses.

Table 2. Sites classes for Montana stream macroinvertebrates.

Site Class	Description
Mountains	In the catchment of the site, the dominant ecoregions are the Northern Rockies, the Idaho Batholith, the Middle Rockies, or the Canadian Rockies, excluding those sites in the Middle Rockies that meet the criteria for Low Valleys. The ecoregions (15, 16, 17, and 41) are generally mountainous.
Low Valleys	In the catchment of the site, the dominant ecoregion is the Middle Rockies (ecoregion 17), the site elevation is lower than 1700 m, the site receives less than 700 mm precipitation per year, and the site has a maximum air temperature greater than 11.0 C (similar to the previously defined Mountain Valleys and Foothills).
Plains	In the catchment of the site, the dominant ecoregions are the Northwestern Glaciated Plains and the Northwestern Great Plains (ecoregions 42 and 43).

4.2.1 Methods

All richness metrics (e.g., insect taxa or non-insect taxa) were calculated such that only unique taxa are counted at the appropriate OTU level. Those taxa that were identified at higher taxonomic levels because of damage or under-developed features were not counted as unique taxa if other individuals in the sample were identified to a lower taxonomic level within the same sample.

Metrics that are calculated based on taxonomic attributes used those attributes assigned by Montana DEQ for functional feeding groups, habit, voltinism, and tolerance. Tolerance metrics were based on Hilsenhoff tolerance values, a scale that ranges from 0 to 10, with sensitive taxa at the 0 end of the range.

Discrimination efficiency

Discrimination efficiency (DE) is the capacity of the biological metric or index to correctly detect stressed conditions. It is measured as the percentage of degraded sites that have values lower than the 25th percentile of reference values (Stribling et al. 2000). For metrics that increase with increasing stress, DE is the percentage of degraded sites that have values higher than the 75th percentile of reference values. DE can be visualized on box plots of reference and degraded metric or index values with the inter-quartile range plotted as the box (Figure 3). When there is no overlap of boxes representing reference and degraded sites, the DE is greater than 75%. The 25th and 75th percentiles were selected for calculating DE because they are easy to conceptualize and have precedence in earlier studies of overlapping distribution (Barbour et al. 1996). Other percentiles were considered for index evaluation and selection of thresholds based on confidence in reference or stressed site selection. A metric with a high DE has a greater ability to detect stress than a metric with a low DE. For this analysis, metrics with DE <25% do not discriminate and were not considered for inclusion in the index.

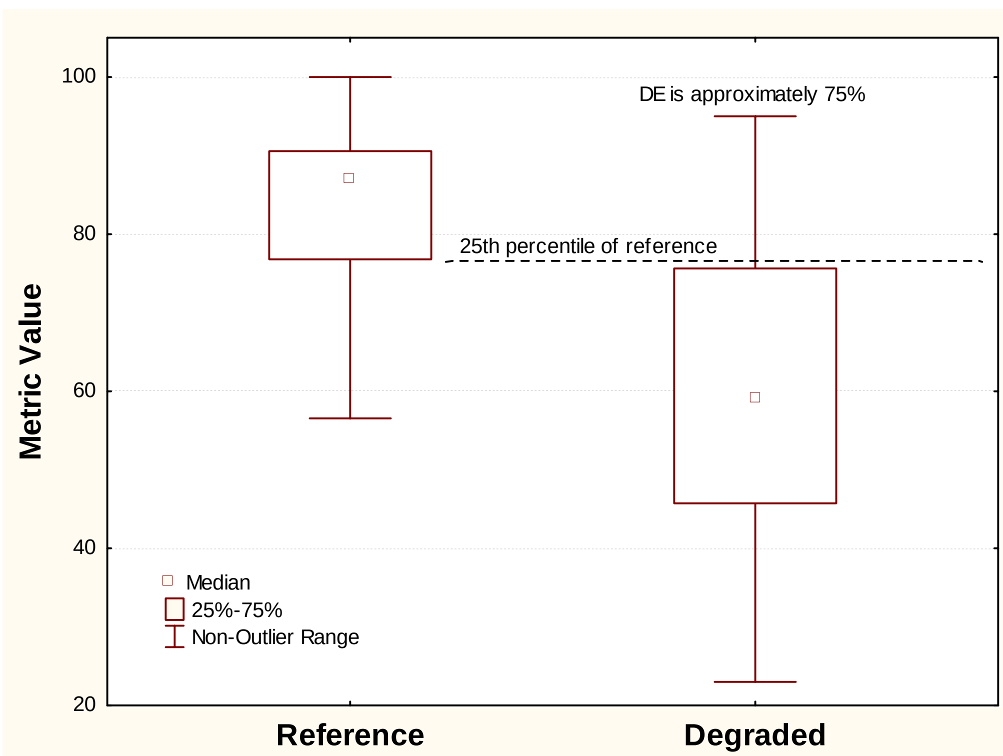

Figure 3. Box and whisker plot illustrating a metric that decreases with increasing stress and that has a DE slightly greater than 75%.

Metric variability

Metric variability was estimated for the reference site population. The coefficient of variability (CV) standardizes variability as a function of mean values (CV = 100* standard deviation / mean). When comparing metrics, those with lower variability in the reference conditions are preferable to those with higher variability. Lower CVs indicate lower variability in relation to means. There was no threshold CV above which metrics would not be included in the index, but metrics with low CVs were preferred over those with high CVs.

Other metric considerations

Ecologically meaningful metrics are those for which the assemblage response mechanisms are understandable and are represented by the calculated value. Ecological meaningfulness is a professional judgment based on theoretical or observed response mechanisms. Those metrics that respond in Montana according to expectations established in other studies are easily defensible. Metrics that show a strong response, but for which we do not have an understandable mechanism were down-weighted, though not entirely excluded. There may be responses in this data set that are not readily explained, but that are justifiable simply because they respond consistently to stress.

Metrics contribute information representative of integrity if they are from diverse metric categories. As many metric categories as practical should be represented in an index so that signals of various stressors can be integrated into the index (Karr and Chu 1999). While several metrics should be included to represent biological integrity, those that are included should not be redundant with each other. Redundancy was evaluated using a Pearson Product-Moment correlation analysis.

For metrics to discriminate on a gradient of stress, they must have a sufficient range of values. Metrics with limited ranges (e.g., richness of taxa poor groups or percentages of rare taxa) may have good discrimination efficiency. However, small metric value changes will result in large and perhaps meaningless metric scoring changes.

Whenever possible, the index is developed, or calibrated, using a subset of all the data. The effectiveness of the indices at distinguishing reference from degraded sites is verified using a separate, preferably independent, data set. After selecting one sample for each site where multiple samples were collected, a second selection process was used to randomly identify calibration and verification sites, within site classes. Metric analyses proceeded using the calibration data only.

4.2.2 Metric Results

We attempted to identify 20% of sites for verification, stratified by reference status and site class (Table 3). In the Low Valleys, initial results from a small verification data set were unsatisfactory, but suspected to be spurious because of the small number of samples used for verification (two reference and four degraded). To develop an acceptable index using the available data, it was decided to forego verification in categories with less than 5 verification samples, using all data for calibration in the Low Valley and Mountain stressed site classes. Verification can be performed as new data are collected in these categories.

Table 3. Sample size by reference class, site class, and calibration/verification status

	Mountains		Low Valleys		Plains	
	Reference	Stressed	Reference	Stressed	Reference	Stressed
Calibration	51	11	13	17	21	24
Verification	14	0	0	0	6	7

Sites and samples may have been excluded from the multimetric analysis for the following reasons. To reduce redundancy in assessing metric responses, sites were excluded if they were within one kilometer of another valid reference site. Sites were also excluded if the site class was undetermined because environmental characteristics were not available due to restrictions in the GIS delineation process (e.g., questionable coordinates). Samples were excluded if fewer than 200 organisms were collected because metrics from such small samples can give inconsistent results.

A total of 111 metrics were calculated using EDAS queries of the macroinvertebrate data reduced to the standard OTU level and their associated taxonomic attributes. DE of each metric was calculated within the site classes (Appendix C). Most metrics were calculated with midge taxa condensed to the subfamily level because it was the lowest level identified in one of the data sets – that from the USFS. A few metrics were calculated with midges at genus level in the Plains region, where USFS samples were limited.

In the Mountain site class, several metrics from each metric category had DEs greater than 50% and a few were greater than 85%. Two metrics, EPT Taxa Percent and Burrower Taxa Percent, had DEs of 100% - all degraded samples had values less than the 25th percentile of reference values. Metrics in this site class generally performed as expected in terms of their trends with increasing levels of stress.

In the Low Valley regions, there are metrics with DEs greater than 50% in all categories. The most responsive metrics were related to midges, which decline in abundance with increasing stress. The strongest response in the tolerance metrics was opposite of expectations. Percent tolerant individuals decreased with increasing stress. The voltinism metrics also responded opposite of expectations, with more short lived organisms in the reference sites than the degraded sites.

In the Plains region, there was at least one metric with a DE greater than 50% in all metric categories. The metrics with the highest DEs were EPT taxa, predator taxa, and percent predator individuals. Of the habit metrics, only % Climbers had a DE greater than 50%. Tolerance metrics did not show strong response to stress, with the two strongest metrics (tolerant taxa and percent super-tolerant) having direction of change opposite to expectations.

4.3 Index Composition

A multimetric index is a combination of metric scores that indicates a degree of biological stress in the stream community (Barbour et al. 1999). Individual metrics are candidate for inclusion in the index if they:

- discriminate well between reference and degraded sites;
- are ecologically meaningful (mechanisms of responses can be explained);
- represent diverse types of information (multiple metric categories); and
- are not redundant with other metrics in the index.

Several index alternatives were calculated using an iterative process of adding and removing metrics, calculating the index as an average of the metric scores, and evaluating index responsiveness. The first index alternatives included those metrics that had the highest DEs within each metric category. Subsequent index alternatives were formulated by adding, removing, or replacing one metric at a time from the initial index alternatives that performed well. The index alternatives recommended for the site classes in Montana met the criteria listed above and could not be improved (increased DE) by substituting, adding, or removing metrics.

Each alternative index was evaluated based on DE (calculated as for individual metrics), separation of reference and degraded index means, and inclusion of representative and unique metrics. Metrics contribute information representative of integrity if they are from diverse metric categories. As many metric categories as practical should be represented in an index so that signals of various stressors can be integrated into the index. While several metrics should be included to represent biological integrity, those that are included should not be redundant with each other.

Redundancy was evaluated using a Pearson Product-Moment correlation analysis. Redundancy can be evaluated such that a threshold for exclusion is established prior to selecting metrics. Thresholds are usually established at coefficient of correlation levels no lower than 0.60 and as high as 0.90 (U.S. EPA 1998). Greater redundancy among metrics in an index is usually avoided because one of the redundant metrics in the set is not contributing new information to the index. There have been arguments to include redundant metrics based on differences in the shapes of response curves (Karr 1991), increased ability to diagnose the causes of degradation (Karr et al. 1986), conceptual differences in the biological significance of the metrics, or a paucity of responsive non-redundant metrics. In this index development effort, we excluded metrics that were redundant at the 0.85 level, except in rare circumstances.

Metrics were scored on a common scale prior to combination in an index. The scale ranges from 0 to 100 (as in Hughes et al. 1998, and Barbour et al. 1999) and the optimal score is determined by the distribution of data. For metrics that decrease with increasing stress, the 95th percentile of all data within the site class was considered optimal (to lessen the influence of outliers [Barbour et al. 1999]), and scored as 100 points using the equation:

$$Score = 100 \times \frac{MetricValue}{95^{th}\ Percentile}$$

All other metric values were scored as a percentage of the 95th percentile value (Figure 4) except those that exceeded 100, which were assigned a score of 100. The 95th percentile value was selected as optimal instead of the maximum so that outlying values would not skew the scoring scale. Metrics that increase with increasing stress (reverse metrics) were scored using the 5th percentile of data as the optimal, receiving a score of 100. Decreasing scores were calculated as metric values increased to the 95th percentile using the equation:

$$Score = 100 \times \frac{95^{th}\ Percentile - MetricValue}{95^{th}\ Percentile - 5^{th}\ Percentile}$$

In some cases, percentiles other than the 95th were used in the equation above to reduce the effects of a skewed distribution.

Index variability can be assessed using the CV, as described for metric variability. In addition, the 90% confidence interval around an observation can be calculated using the formula:

$$90\%CI = \frac{StdDev \times 1.64}{\sqrt{n}},$$

where the standard deviation (*StdDev*) is derived from repeated measures at a site. As more measures are taken, the confidence interval shrinks. The confidence interval is used to enhance the interpretation of observed index values in relation to other index values or a threshold. It should not be used to discount unexpected results, saying that the true mean could be closer to the expected value by as much as the confidence interval. Rather, it can be used to identify observations that may require continued monitoring because they contain a threshold (or a comparable site observation) within the confidence interval.

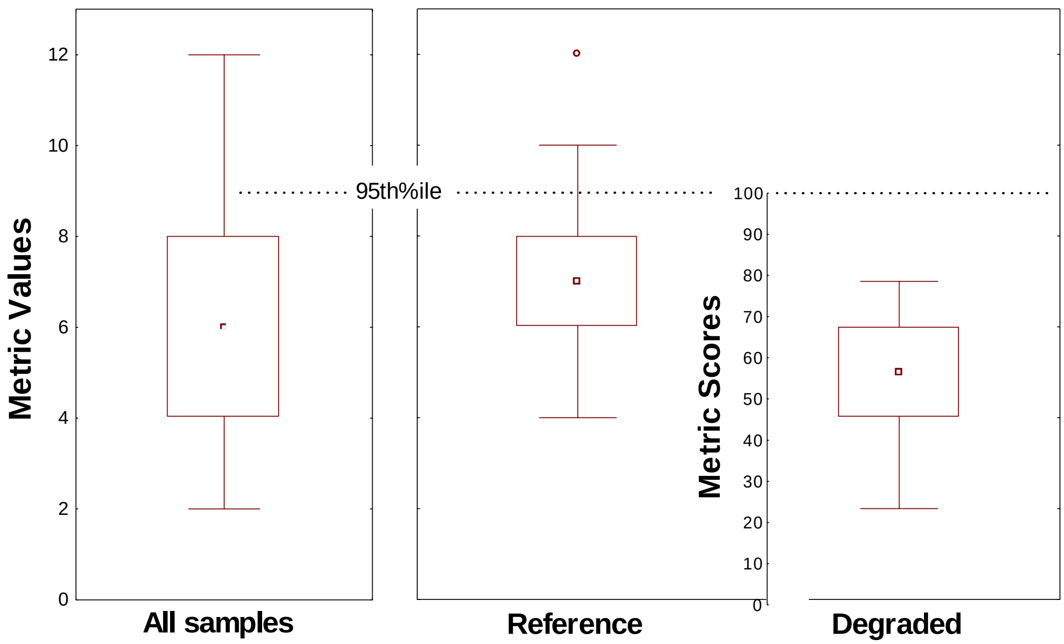

Figure 4. Schematic example of the scoring scale for metrics that decrease with increasing stress.

4.3.1 Index Composition Results

One hundred and four (104) index alternatives were calculated and tested, at least 16 in each site class (Appendix D). The index alternatives that performed best in each site class were reconsidered and the following three indices were recommended.

Mountain Index

In the mountainous and higher elevation regions, 15 index alternatives included understandable metrics and performed equally well in terms of the DEs – 100% for calibration data. The recommended index has good separation between the means of reference and degraded index values. The other alternatives are less attractive because they use somewhat less favorable metrics. For example the Coleoptera taxa increase with increased stress, but have a relatively limited possible range (up to 7 taxa). The percent EPT taxa decreases with increasing stress, but includes the Trichoptera, which do not respond well as a group. The recommended index includes metrics from five of the six main categories (richness, composition, trophic behavior, habit, voltinism, and tolerance). Ideally, all six categories are represented, but the lack of adequate discriminatory metrics in the voltinism category prohibited their representation. The index alternative that is recommended for adoption in the Mountains contains seven metrics, as follows:

- Ephemeroptera Taxa — Score = 100****X***/10
- Plecoptera Taxa — Score = 100* ***X*** /7
- % EPT — Score = 100* ***X*** /90
- % Non-Insect — Score = 100*(28- ***X***)/28

- % Predator   Score = 100* ***X*** /39
- Burrower Taxa Percent   Score = 100*(83- ***X***)/71
- Hilsenhoff's Index   Score = 100*(7.5- ***X***)/6

Among the seven metrics in the Mountain Index, the two most highly correlated are % EPT and the Hilsenhoff Index, with a correlation coefficient of –0.69 (Table 4). This redundancy is mostly due to the fact that EPT taxa dominate mountain streams in Montana, but we consider it acceptable since the index metrics give somewhat independent signals of biological stress.

Table 4. Correlations (Pearson Product-Moment) among metrics of the Mountain Index.

	Ephemerop. Taxa	Plecoptera Taxa	% EPT	% Non-Insect	% Predator	Burrower Taxa %
Plecoptera Taxa	0.47					
% EPT	0.37	0.36				
% Non-Insect	-0.32	-0.23	-0.51			
% Predator	0.01	0.11	-0.27	0.29		
Burrower Taxa %	-0.51	-0.51	-0.53	0.26	-0.04	
HBI	-0.44	-0.55	-0.69	0.55	0.25	0.53

All of the index alternatives performed better than the Mountain IBI previously used by Montana DEQ, which had a DE of 81.8% (Appendix D). In this data set, three of the historically applied metrics (total taxa, % dominant, and % scrapers and shredders) had DEs lower than viable alternatives (Appendix C).

Mountain Index Interpretation

The metrics in the Mountain index are fairly straightforward in interpretation. Although the mechanisms by which aquatic macroinvertebrates responded to environmental stressors may not be fully understood (adequate environmental data and mechanistic information is often lacking), the fact that the metrics were responsive to a general gradient of stress (reference – degraded) (see Appendix C) suggests that they were responding to a common suite of stressors. The metrics in this and the other indices were therefore selected largely based on their demonstrated responses in this data set.

Ephemeroptera and Plecoptera taxa (mayflies and stoneflies) are generally sensitive to environmental degradation such as reduced dissolved oxygen, unstable substrates, and contamination due to heavy metals and other toxicants. As environmental conditions become worse, the sensitive and specialist taxa of these groups will emigrate or perish. This effect is paralleled in the Percent EPT metric, in which sensitive and specialist *individuals* of the mayfly, stonefly, and caddisfly insect orders will emigrate or perish. Non-insects (primarily gastropods, bivalves, crustaceans, and worms) are generally tolerant of habitat stresses that cause greater sedimentation and are able to take advantage of a variety of food sources such as detritus, suspended organic material, and epibenthic algae. Their increase in stressed conditions reflects an increase of these food sources or benthic sediments compared to reference conditions.

Predator individuals decrease with increasing stress, perhaps also due to shifts in food resources and an increase in collectors and filterers. As stress increases in the Mountains, Burrowers are more prevalent in the taxa lists, perhaps indicating habitat conditions with greater amounts of fine sediments. Hilsenhoff's Index increases with stress, indicating that individuals tolerant of pollution inhabit the degraded streams.

Based on field replicates using the traveling kick sampling method, the Mountain Index has a CV of 6.4% and a 90% confidence interval of ±6.9 index points for a single observation. These statistics describe a variability that is well within our expectations of an index that can discern site to site differences of 20 points.

Low Valley Index

In the Low Valley regions, the recommended index has a DE of 94%. It contains five metrics, representing two of the six metric categories, as follows:

- % EPT excluding Hydropsychidae and Baetidae Score = 100****X***/71
- % Chironomidae Score = 100****X***/40
- % Crustacea & Mollusca Score = 100*(20- ***X***)/20
- Shredder Taxa Score = 100*(7- ***X***)/7
- % Predator Score = 100****X***/33

Index alternatives with metrics from the richness, habit, voltinism, and tolerance categories did not perform as well as alternatives without them. Metrics from these categories either had low DEs or responded in a direction contrary to our understanding – short-lived and pollution tolerant organisms were more prevalent in the reference streams. Among the five metrics in the Low Valley Index, the two most highly correlated are Percent Chironomidae and Percent Predator, with a correlation coefficient of 0.41 (Table 5). This degree of redundancy is acceptable, showing that the index metrics are giving independent signals of biological stress.

All alternative indices in the Low Valley site class out-performed the Mountain Valley and Foothill index currently used by DEQ (which had a DE of 17.6%) (Appendix D). None of the metrics in the Mountain Valley and Foothill Index performed well with this data set.

Table 5. Correlations (Pearson Product-Moment) among metrics of the Low Valley Index.

	% EPT excluding Hydro. & Baet.	**% Chironomidae**	**% Crustacea & Mollusca**	**Shredder Taxa**
% Chironomidae	-0.34			
% Crustacea & Mollusca	-0.18	-0.17		
Shredder Taxa	0.16	-0.15	0.09	
% Predator	0.04	0.41	-0.06	0.07

Low Valley Index Interpretation

The Low Valley Index contains the Percent EPT metric, excluding the somewhat tolerant families of Hydropsychidae (Trichoptera) and Baetidae (Ephemeroptera). This metric has the lowest DE of all those in the index (DE = 53%), but is included because the responses of these taxa have ample precedent in biomonitoring assessments (Barbour et al. 1999). The index also includes the Percent Chironomidae metric, which decreases with increasing stress. Though the ecological mechanism of this response is not well understood at this point, the signal is very strong in this data set (DE = 70.6%) and the metric was deemed appropriate for inclusion in the index. The midges are apparently sensitive taxa that disappear with increasing stress. This is confirmed by the Chironomidae Taxa metric, which decreases with increasing stress and has a high DE, especially with midges identified to genus level (Appendix C). This metric was not used in the index because such use would preclude evaluation of Forest Service samples (where midge identifications are only made to the sub-family level). An increase of Crustacea and Mollusca in the Low Valleys is analogous to the increase in Non-Insects observed in the Mountains. These are primarily collectors, scrapers, and filterers that can tolerate fine sediments more so than some other taxa. Shredder Taxa increase with increasing stress, indicating that coarse particulate matter is more available in degraded Low Valley streams compared to the reference streams. Percent Predator individuals was the second best performing metric in the Low Valleys, showing that the functional structure of the assemblage changes considerably with increasing stress.

Based on field replicates using the traveling kick sampling method in Low Valley sites, the index has a CV of 9.1% and a 90% confidence interval of ±8.4 index points for a single observation. These statistics describe a variability that is well within our expectations of an index that can discern site to site differences of 20 points.

Plains Index

Index development for the Plains of Montana was an iterative process. Initial indices appeared to have acceptable DEs for the calibration data, but the verification degraded sites were indistinguishable from reference. It was assumed that this was a coincidental result, which can occur when a small verification subset is randomly selected. This led to a rejection of the initial models and model redevelopment based on new random assignments of all Plains samples into calibration and verification subsets. It followed that new metric DEs were calculated, and new indices were tested based on metrics that had high DEs in both the initial and the second subsets of calibration samples. The final selection of an index was based on the best index performances in both the initial and secondary calibration data sets.

In the Plains regions, the recommended index has five metrics, as follows:

- EPT Taxa — Score = 100****X****/14
- % Tanypodinae — Score = 100****X****/10
- % Orthocladiinae of Chironomidae — Score = 100*(100-***X***)/100
- Predator Taxa — Score = 100****X****/9
- % Filterers and Collectors — Score = 100*(100-***X***)/65

The DE of the initial calibration subset was 92% and the DE of the secondary calibration subset was 75%. Statistics to describe the accuracy of the Plains MMI should be derived from the secondary data set, which showed adequate verification (see Section 4.3.2). The DE for all secondary Plains data (calibration and verification combined) was 77.4%.

Among the five metrics in the Plains Index, the two most highly correlated are Predator Taxa and percent Filterers and Collectors, with a correlation coefficient of -0.32 (Table 6). This degree of redundancy is acceptable, showing that the index metrics are giving independent signals of biological stress. Index DE could not be improved by adding metrics based on midge identifications at genus.

Table 6. Correlations (Pearson Product-Moment) among metrics of the Plains Index.

	EPT Taxa	% Tanypodinae	% Orthoclad. of Chir.	Predator Taxa
% Tanypodinae	-0.18			
% Orthocladiinae of Chironomidae	0.27	-0.23		
Predator Taxa	-0.11	0.15	-0.27	
% Filterers and Collectors	0.23	-0.30	0.26	-0.32

In this data set, the Plains MMI out-performed all of the historically applied indices that were tested (Appendix D). This includes the index currently used by DEQ as developed by Bramblett and others (2003) for riffle samples, a companion index for Prairie pool samples (Bramblett et al. 2003), the Prairie index developed by Marshall and Kerans (2003), and an index for the basin and plains areas of Wyoming by Stribling and others (2000).

Plains Index Interpretation

EPT taxa are generally sensitive to pollution (Barbour et al. 1999), and they tend to become less diverse as stresses increase in the Plains. As in the Low Valleys, certain midges in the Plains appear to be sensitive to stress. The Tanypodinae decrease in relative abundance with increasing stress while the relatively tolerant Orthocladiinae increase as a percentage of all Chironomidae. Percent Filterers and Collectors increase with increasing stress, which may indicate that the food resource or substrate changes from attached algal turf to detritus and suspended solids. The Predator Taxa metric decreases with increasing stress, which also indicates functional changes in the assemblage.

Based on field replicates using the traveling kick sampling method in Plains sites, the MMI has CV of 16.8% and a 90% confidence interval of ±9.6 points on a 100 point scale. These statistics describe a variability that is within our expectations of an index that can discern site to site differences of 20 points.

4.3.2 Index Verification

The indices developed for the Mountain and Plains used a subset of the data, reserving an independent subset of sites for verification of the index. A robust index will perform as well or nearly as well with an independent data set, showing comparable DEs and similar response patterns. The indices were developed to perform optimally with the calibration data. We can expect some decline in performance with verification data. The acceptable degree to which verification results resemble calibration results is somewhat subjective.

In the Mountain site class, 71% of verification reference sites were above the 25th percentile of calibration reference values (Figure 5). These results indicate that the index is robust and performs well at identifying reference-quality samples in an independent data set. For equal performance, 75% of reference sites would have index values greater than the calibration reference 25th percentile. Index performance in degraded sites was not performed because all the available data were used in calibration.

The Low Valley index was not verified because all of the data were used in calibration. The decision to use all data for index calibration was reached after attempts to calibrate and verify an index were unsuccessful. The failure was attributed to small sample sizes and better results were expected from a larger calibration data set. The index performs well with all the calibration data, though one reference site, Landslide Creek within Yellowstone National Park, scores quite low (Figure 6).

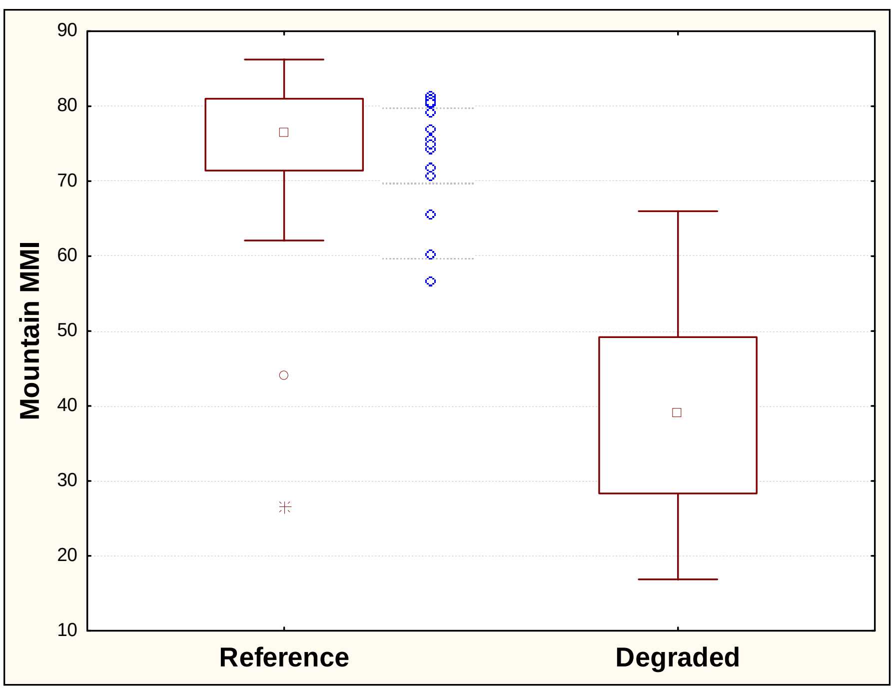

Figure 5. Distributions of the Mountain MMI in reference and degraded sites. Box and whisker plots represent calibration data and open circles represent individual verification data points for reference sites. In degraded sites, all data were used for calibration.

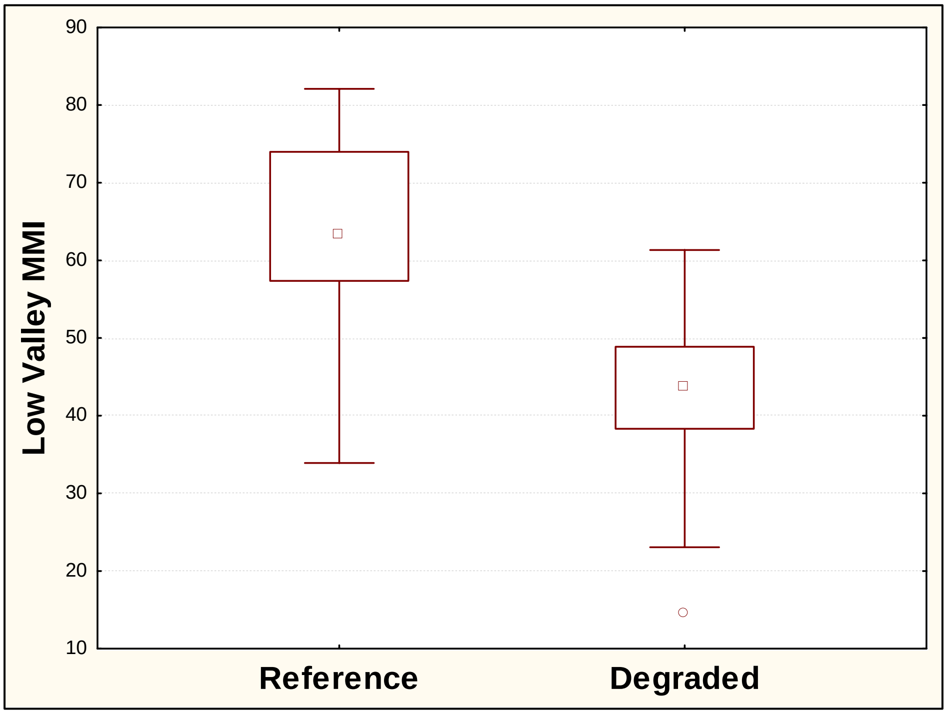

Figure 6. Low Valley MMI showing distributions of values by reference status

In the Plains verification process, all verification samples scored well using the initial calibration and verification data sets (Figure 7). In this data set, the degraded verification samples all had high MMI scores. Thus, the model was not verified for degraded samples. For reference samples, 4 of 5 samples were greater than the calibration reference 25th percentile and the model was verified for reference samples. The secondary calibration and verification data sets show better verification in degraded sites (Figure 8). Compared to the calibration reference 25th percentile, 4 of 6 (67%) verification reference samples were above and 5 of 7 (71%) verification degraded samples were below.

4.3.3 Conclusions

Three indices are recommended for bioassessment of streams based on macroinvertebrate samples (Table 7). These indices are specific to the site class in which a site belongs, and have been calibrated using the dataset available. They are believed to be improvements over previously applied indices because of the rigor with which reference and degraded sites were identified, the scientific process used for identifying responsive metrics and indices, and the quantification of the index accuracy (DE) and precision (90% confidence interval).

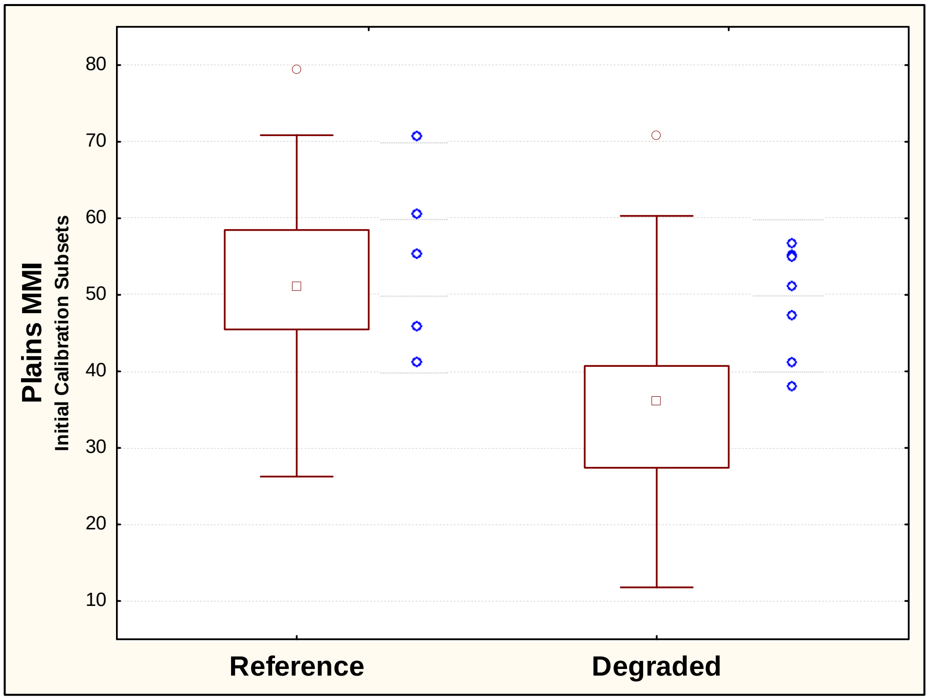

Figure 7. Distributions of the Plains MMI in reference and degraded sites for the initial calibration and verification subsets. Box and whisker plots represent calibration data and open circles represent individual verification data points.

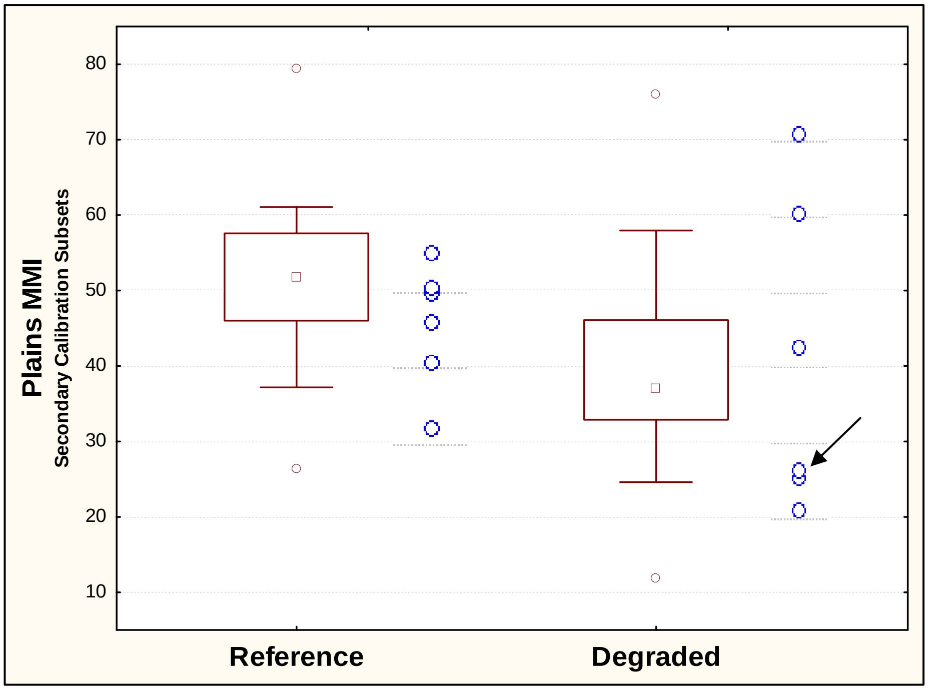

Figure 8. Distributions of the Plains MMI in reference and degraded sites for the secondary calibration and verification subsets. Box and whisker plots represent calibration data and open circles represent individual verification data points. One verification degraded data point is hidden so that the lower grouping represents four samples (see arrow).

Table 7. Metrics in the recommended indices for three site classes in Montana, showing metric response with increasing stress (- : decreasing, + : increasing).

Mountain Index	Low Valley Index	Plains Index
Ephemeroptera Taxa (-)	% EPT excluding Hydropsychidae and Baetidae (-)	EPT Taxa (-)
Plecoptera Taxa (-)	% Chironomidae (-)	% Tanypodinae (-)
% EPT (-)	% Crustacea & Mollusca (+)	% Orthocladiinae of Chironomidae (+)
% Non-Insect (+)	Shredder Taxa (+)	Predator Taxa (-)
% Predator (-)	% Predator (-)	Filterers and Collectors (+)
Burrower Taxa Percent (+)		
Hilsenhoff's Index (+)		

The Mountain Index is the most robust of the three indices, having a high DE and adequate verification in reference sites. The Mountain Index also uses metrics that are easily understood, mostly have precedent uses in indices of Montana or surrounding areas, and have excellent discrimination ability individually. The index recommended here has a DE of 100% and a 90% confidence interval of ±6.9 index units. It out-performs the index currently applied by Montana DEQ and should replace it.

The Low Valley Index recommended here out-performs the index currently applied by DEQ in Mountain Valleys and Foothills. DE is high for all the data, at 94%, though the model has not been verified. Model verification should be performed as new data are collected. The 90% confidence interval is ±8.4 index units. This index includes five metrics from three metric categories. None of the richness, voltinism, or tolerance metrics effectively responded to stress in this analysis. Nonetheless, this index is the best indicator of macroinvertebrate assemblage integrity and should be used for the Low Valley streams of Montana.

The Plains Index is the weakest among the site classes, with a DE of 77.4%. Though the index DE suggests a relatively high level of uncertainty regarding site assessment, the error rate is at least quantifiable and can be explicitly stated along with any assessments that depend on it. This index out-performs other indices used by DEQ in this site class (which do not have quantifiable error rates). The 90% confidence interval is ±9.6 index units.

After calibrating and verifying the indices in the Mountain and Plains site classes, calibration and verification data were combined to describe the distributions of index values in all reference and degraded sites. Montana DEQ should take the next step of establishing threshold index values upon which to base aquatic life use attainment determinations. These thresholds, or biocriteria, should take uncertainty and error into account.

One indication of uncertainty is the accuracy of the index – the DE. In the Mountain Index, accuracy is good and a relatively low threshold may be chosen to balance error rates among reference and degraded sites of this data set (Table 8). In the Plains, however, the index does not discriminate as well as in the Mountains. This may be an indication of high variability in the reference condition or stresses that do not induce strong biological responses. When setting thresholds in the Plains, policy may dictate a need to allow greater error on one or the other side of the threshold. The rigor with which the reference site database was developed (Suplee et al. 2005) could potentially justify selecting a lower threshold, such as the 10th percentile.

Table 8. Index statistics useful for establishing biocriteria or thresholds, based on calibration and verification data combined.

Reference Percentile	Min	10th	25th
Mountain Index			
Index Value	26.6	63.5	71.4
% Degraded Below	18.2	**90.9**	100
% Reference Above	100	**90**	75
Low Valley Index			
Index Value	33.9	48.1	57.4
% Degraded Below	23.5	70.6	94
% Reference Above	100	90	75
Plains Index			
Index Value	26.3	37.2	45
% Degraded Below	19.4	54.8	**77.4**
% Reference Above	100	90	**75**

Bold type indicates approximately equal error rates in reference and degraded sites.

A second indication of uncertainty is sampling error, which can be described using analysis of replicated measures. The 90% confidence interval around the three recommended indices ranges from 6.9 to 9.6 index points. An observation on one side or the other of a selected threshold should be designated as passing or failing the threshold based on the observation. However, if the threshold is within the 90% confidence interval of the observation, the site should be repeatedly monitored to allow placement in the passing or failing category with greater confidence, keeping two things in mind: 1) that with greater repetition of measurements, the confidence interval will shrink to less than 10 points, and 2) the uncertainty associated with the threshold (Table 8) will still apply.

Still, a third consideration to be taken when applying the index regards the certainty of site class membership. When more than 80% of the catchment of a site is within a clearly defined ecoregion, then class membership is fairly straightforward. However, those sites that have catchments split evenly over the ecoregional boundaries, or that meet some, but not all of the criteria for Low Valleys, may be placed in a site class with less certainty. In these uncertain cases, it may be appropriate to apply indices from both site classes and determine whether the results agree or disagree.

Finally, the issue of sample collection methods was examined recently (Jessup et al. 2005), concluding that assessment using different protocols will essentially result in similar outcomes. This should be confirmed using the comparability study data and the newly calibrated indices, and should be expanded to include more samples from the Plains, where the previous study did not focus. Until that confirmation, efforts should be made to standardize sample collection protocols to reduce variability that multiple methods may introduce.

Also, as an overall caution on the entire calibration process, evaluating the ability of individual metrics to detect specific conditions from individual stressor types (e. g., nutrient enrichment) is directly reliant upon the existence of sites bearing those characteristics. As an illustration, the stressor site dataset for the Low Valley site class did not have known nutrient-enriched sites well represented. Therefore, the ability of some Low Valley metrics to detect the effects of those site stressors may be suboptimal.

Index application should proceed as follows:

1) Collect data and organize them in EDAS.
2) Determine the appropriate site classes, using criteria in Table 2.
3) Calculate metrics and indices using functions in EDAS or by scoring metrics according to formulas in Section 4.3.1. The rarefaction algorithm must be applied on samples larger than 360 organisms.
4) Derive the index values by averaging the index scores.
5) Compare resulting index scores to established thresholds of impairment and make judgments on aquatic life use attainment, qualifying the judgments with indications of uncertainty.

5.0 Predictive Model Development

Procedures for developing and evaluating RIVPACS models have been well documented and we only describe details germane to the Montana model here (Clarke et al. 1996, 2002, 2003, Hawkins et al. 2000, Hawkins and Carlisle 2001, Ostermiller and Hawkins 2004, Van Sickle et al 2005). Development and evaluation of RIVPACS models require the following steps:

1. Selection of a set of reference sites that adequately represent the naturally occurring environmental gradients in the region of interest (whole state, subregions, etc.).
2. Classification of reference sites based on their taxonomic similarity to one another.
3. Estimation of frequencies of occurrence of each taxon in each reference site class.
4. Development of a discriminant function model to predict the probability of a new site belonging to each reference site class from surrogate variables representing important determinants of taxon distributions.
5. Estimation of taxon probabilities of capture as the frequencies of occurrence among classes weighted by the probabilities of a site belonging to a class.
6. Estimation of the expected number of taxa at a site as the sum of the predicted probabilities of capture.
7. Assessment of the performance of the model by (1) comparing the observed number of predicted taxa (O) found at reference sites with the expected number of taxa (E) and (2) calculation of the precision in O/E estimates.

5.1 Methods

5.1.1 Reference Sites Used for Modeling

One hundred and thirty seven reference quality samples were available for model building. Of these samples, 112 were collected from reasonably spatially unique sites. Of these 112 samples, 104 contained at least 200 individuals, which was the minimum (200-300) selected for model building. One of these 104 sites was influenced by a hot springs and was dropped from modeling. Of these 103 reference sites, 27 were sampled by the state of Montana or its contractors, 23 were sampled by the US Forest Service, 17 were sampled by the EMAP program of the US EPA, and 37 were sampled by the Utah State University STAR project (Figure 9).

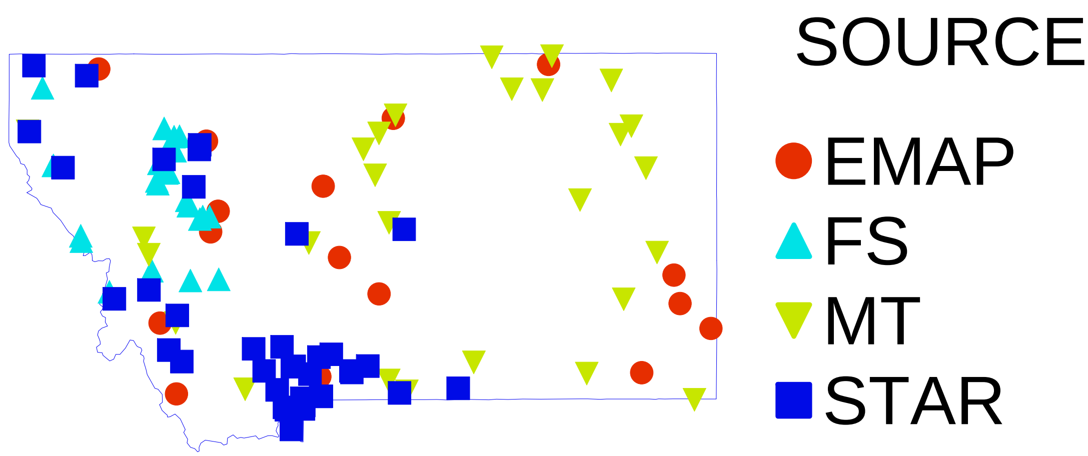

Figure 9. Location of 103 reference sites and source of samples.

5.2 Results

5.2.1 Classification and Site Grouping

One hundred and seventy seven OTUs (operational taxonomic units) were found in the samples collected from the 103 unique reference sites used to build the model. Eighty-eight OTUs were observed in 5 or more samples and were used to create the biotic classification of sites on which the predictive model was based (Appendix E). A classification dendrogram was produced by first calculating all pair-wise Bray-Curtis similarities between samples and then clustering sites with the flexible-beta UPGMA algorithm (McCune and Grace 2002). Six groups of sites were identified from this dendrogram (Figure 10), of which one (2b) contained too few sites for modeling. It was subsequently combined with group 2a resulting in 5 groups for use in modeling. These 5 groups were subgroups of two distinctly different sets of sites, which generally occurred in lowland and upland regions, respectively (Figure 11). Group 1 clearly represented streams of the eastern plains. Streams in Group 2 occurred in central Montana as well as western valleys that appeared to be spring influenced or transitional streams between ecoregions. Streams in Groups 3-5 occurred in the western mountains, but otherwise showed little spatial structure with respect to their geographic location.

5.2.2 Discriminant Models

We used the all subsets software developed by John Van Sickle of the USEPA (Corvallis, OR) to select the discriminate model that most effectively minimized bias and maximized precision of model predictions. This software evaluates up to 32,767 models based on all possible combinations of 15 or fewer predictor variables. Software output includes the 5 best performing models for each of 1st through 15th -order (predictors) models. Performance measures include the mean, standard deviation, and root mean square error of O/E values derived from reference quality samples. These measures are compared with estimates of the error expected if no natural environmental gradients were accounted for (null model) and a theoretically perfect model in which the only error was the random variation expected among replicate samples (see Van Sickle et al. 2005). Ideally, models are evaluated with an independent set of validation samples collected from a range of reference-quality waterbodies. However, the small number of reference sites prohibited such an external validation. All performance measures reported here are based on internal validation in which the original data were run back through the models.

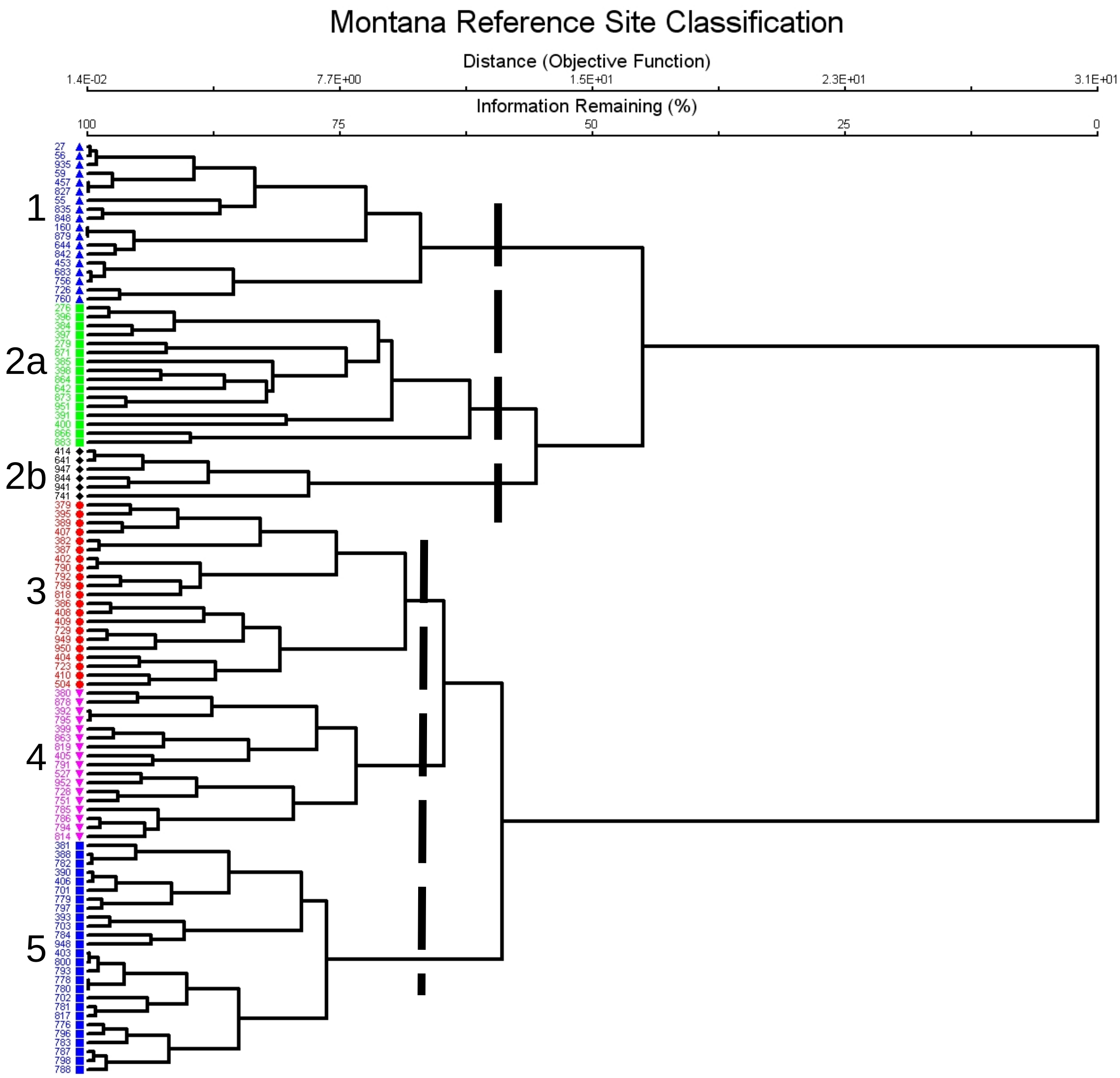

Figure 10. Dendrogram showing the 5 site clusters used in modeling. Dashed lines show the level of within group similarity at which groups were defined. Numbers and colors at the left of the dendrogram indicate group assignment. Groups 2a and 2b were combined for modeling. Two distinct large groups (subgroups 1-2 and 3-5) generally represent lowland and upland streams, respectively.

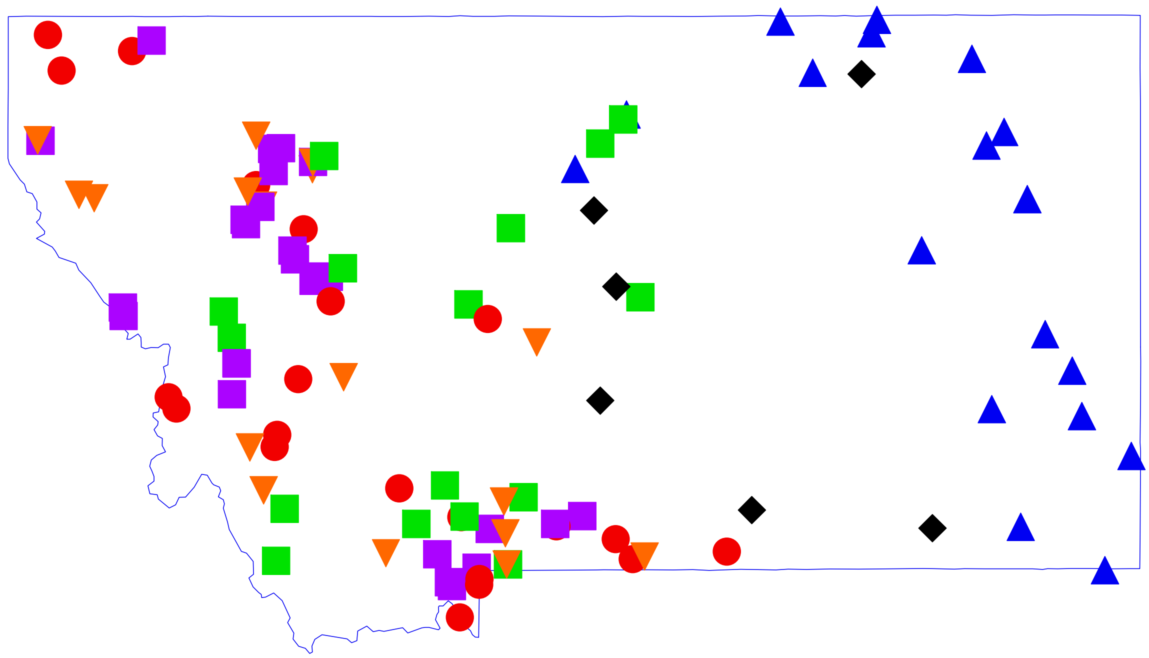

Figure 11. Location of the 103 reference sites classified by biotic groups. Groups 1 (blue triangles) and 2 (2a=green squares, 2b=black diamonds) largely occur in lowland areas, and groups 3 (red circles), 4 (orange triangles), and 5 (purple squares) occur in upland areas. Note the lack of significant spatial clustering of sites in groups 2-5.

5.2.3 Model Performance

We chose to calculate O and E based on a probability of capture threshold of > 0.5. Use of lower thresholds increase the number of taxa on which assessments are based, but they usually result in increased error (lower precision) associated with the prediction of rare taxa (Hawkins et al. 2000, Ostermiller and Hawkins 2004).

The final model used 5 predictor variables: latitude (decimal degrees), longitude (decimal degrees), mean maximum annual air temperature (^{o}C x 10), a dummy variable indicating whether the site was in the Columbia River Basin (CRB) or not (1/0), and log watershed area (km^2). Of these variables, longitude and mean maximum annual temperature varied the most among classes (partial F-values: longitude = 20.45, mean maximum temperature = 14.87, latitude (9.39), CRB (5.79, and log watershed area (5.02), Figure 12). The strong association between biotic class membership and longitude and temperature implies that factors associated with climate and stream gradient were the most important factors affecting the distribution of stream taxa.

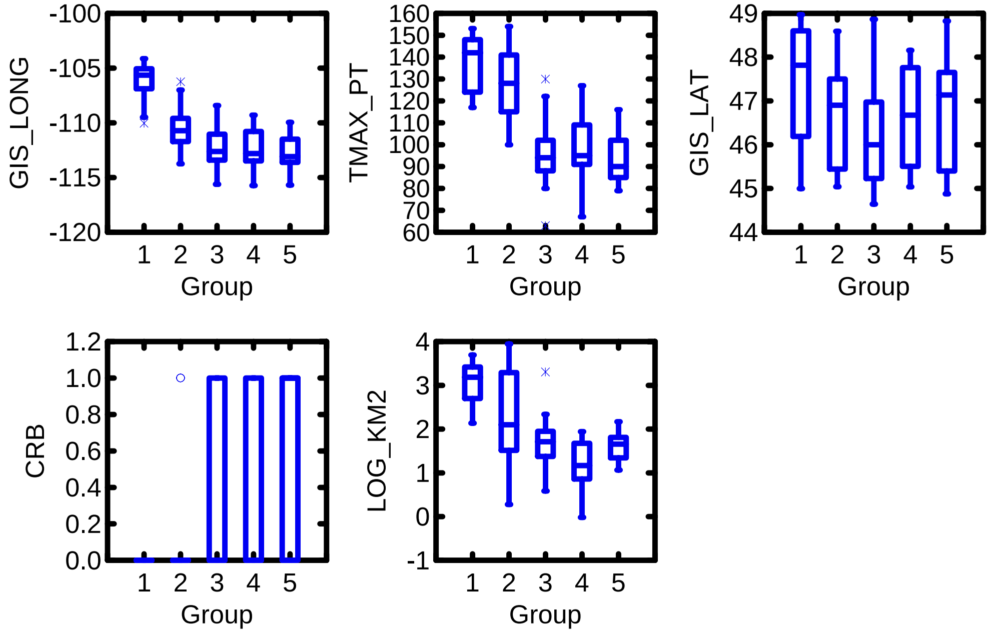

Figure 12. Box-whisker plots showing the variation in longitude, mean maximum annual temperature, latitude, CRB, and log watershed area among and within the 5 biologically defined classes (groups).

The mean O/E value of the calibration sites was 0.99 and the standard deviation was 0.17. This estimate of error was far better than that associated with the null model (0.38) and the model accounted for ~ 88% of the explainable variability in taxonomic composition among samples (Figure 13). Much of the error in the model was associated with variability among group 2 samples. The variability in reference site O/E values for group 2 samples (SD = 0.26) was > twice as much as that observed for most other groups (SD = 0.15, 0.11, 0.12, 0.12 for groups 1, 3, 4, and 5, respectively). O/E estimates were more precise for upland streams than their lowland counterparts.

To be most useful, predictive model assessments need to be accurate as well as precise. In general, the model was accurate in that the slope of the relationship between O and E was not significantly different from 1 (Figure 14), and there was little tendency for the model to over- or under-predict for any of the 5 groups (Figure 15). The model accounted for differences in richness observed both among reference site groups as well as within groups (Figure 14).

The model also showed little evidence that it produced biased predictions for streams that occurred in different regions of Montana as defined either by major ecoregion (Fig. 16a) or dominant geology (Fig. 16b).

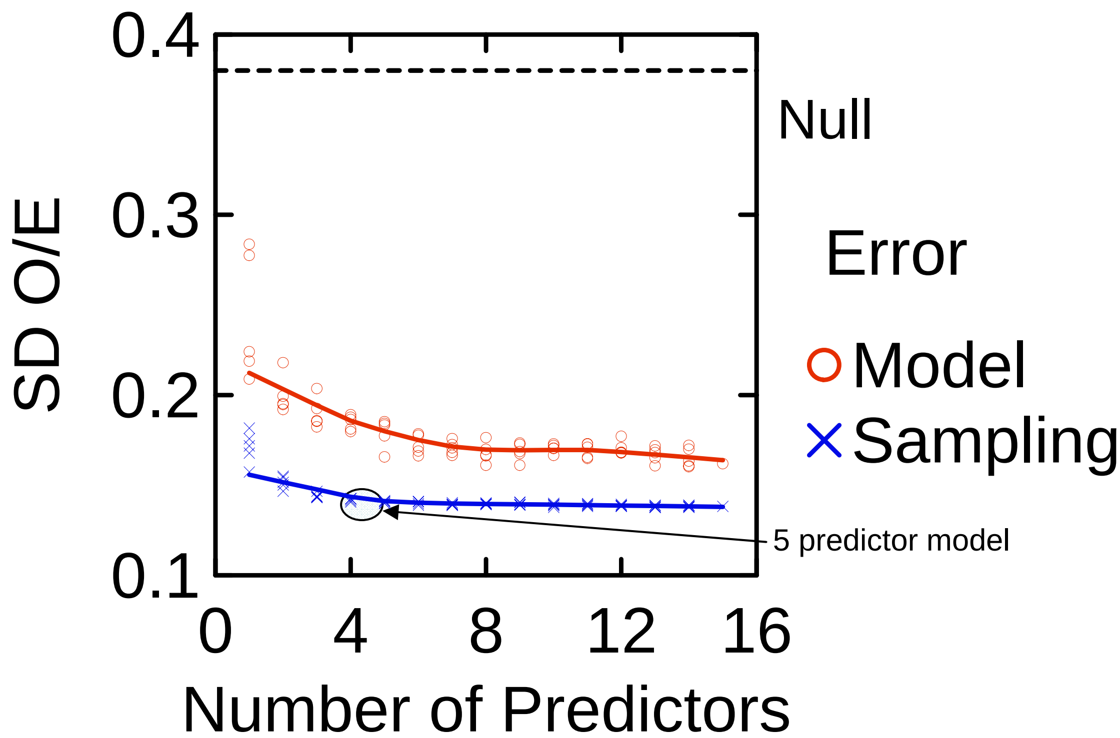

Figure 13. Relationship between model error (SD of reference quality samples) and the number of predictor variables used in the best 71 models. The maximum possible error is given by the null model and the lowest possible error by the estimate of random sampling error. O and E were calculated with a probability of capture threshold of > 0.5.

5.2.4 O/E Sensitivity

Because RIVPACS models predict how taxa should be naturally distributed across sites, if the models are accurate, the only factor that should affect the sensitivity of assessments is the sensitivity or tolerance of the taxa in the region to the stressors that exist. Because the OTUs we used in the models generally represent relatively coarsely resolved taxa (e.g., many genera, some families, a few species), these assessments will be conservative with respect to what we would see with models based on species-level data (Hawkins et al. 2000).

In spite of the fact that OTUs generally represented groupings of more than one species (and thus the response of sensitive species to stress could be masked by less sensitive species lumped in the OTU), O/E values at sites considered to be stressed by chemical or physical factors were generally low (Figure 17). In general, O/E values effectively discriminated the stressed sites from the reference sites, especially for the upland streams in western Montana and the valley streams of the Middle Rockies (Figure 17). There was somewhat less discrimination between reference and test sites for the plains streams of eastern Montana, an observation consistent with the lower precision associated with predicting taxa composition in streams of this region.

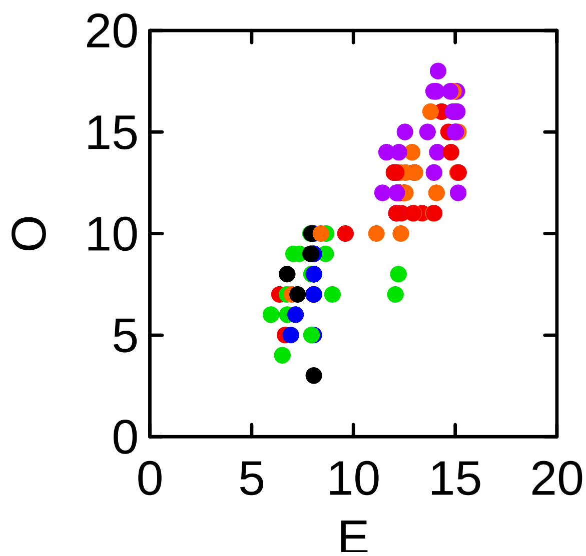

Figure 14. Relationship between observed richness (O) and expected richness (E) at reference sites. The model accounted for 76% of the variation in O and the slope of the relationship (1.04) was not different from 1. O and E were calculated with a probability of capture threshold of > 0.5.

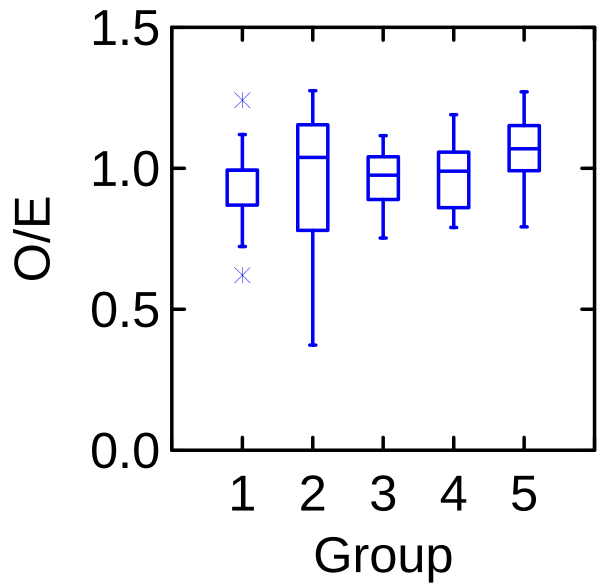

Figure 15. Box-plots of reference site O/E values by classification group. There was little tendency to either over- or under-estimate O/E values based on the biotic class to which sites were assigned, with the possible exception of group 5 for which E may have been slightly under-predicted (mean O/E = 1.08). However, only 8% of the variation in O/E among samples was associated with group.

In general, the performance of the Montana RIVPACS model is comparable to or better than most RIVPACS models in use in the USA and elsewhere in terms of model precision (Hawkins 2006 [*in press*]). Good models typically have O/E standard deviations less than 0.18 in reference sites, as does the one presented here. The fact that the model makes good predictions from just 5 easily derived predictor variables means it will be easy to implement. In spite of the paucity of reference sites in lower elevation regions of Montana, the model appeared to be surprisingly robust in those regions, albeit less precise.

5.3 Model Output and Interpretation

The web software provides 4 output files to aid users in interpreting assessments.

5.3.1 Is the Site Within the Experience of the Model?

An important consideration in bioassessment is that assessed sites be matched as closely as possible to their appropriate reference condition. In general, predictive models accomplish this matching by predicting the taxa that should occur at a site given the values of several predictor variables. However, these predictions can be made with considerable error if models are allowed to extrapolate beyond the range of predictor values used to calibrate models. The USU web software produces a "site test" file in which any site whose combination of predictor values are outside the experience of the model is flagged. O/E values calculated at these sites should be interpreted with caution.

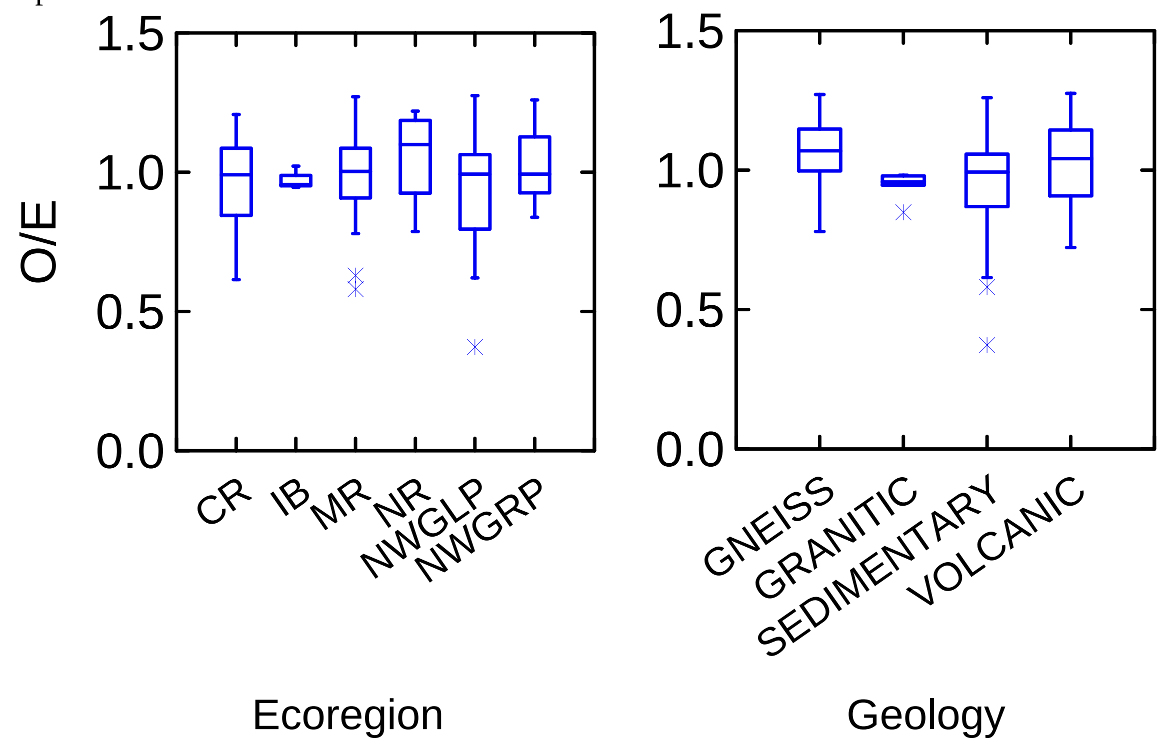

Figure 16. Box plots of reference site O/E values by ecoregion (a) and geology (b). CR = Canadian Rockies, IB = Idaho Batholith, MR = Middle Rockies, NR = Northern Rockies, NWGLP = North West Glaciated Plains, NWGRP = North West Great Plains. Less than 4% of variation in O/E values were associated with ecoregion. About 9% of the variation in O/E values were associated with geology. The model slightly under-estimated E for streams draining gneiss basins.

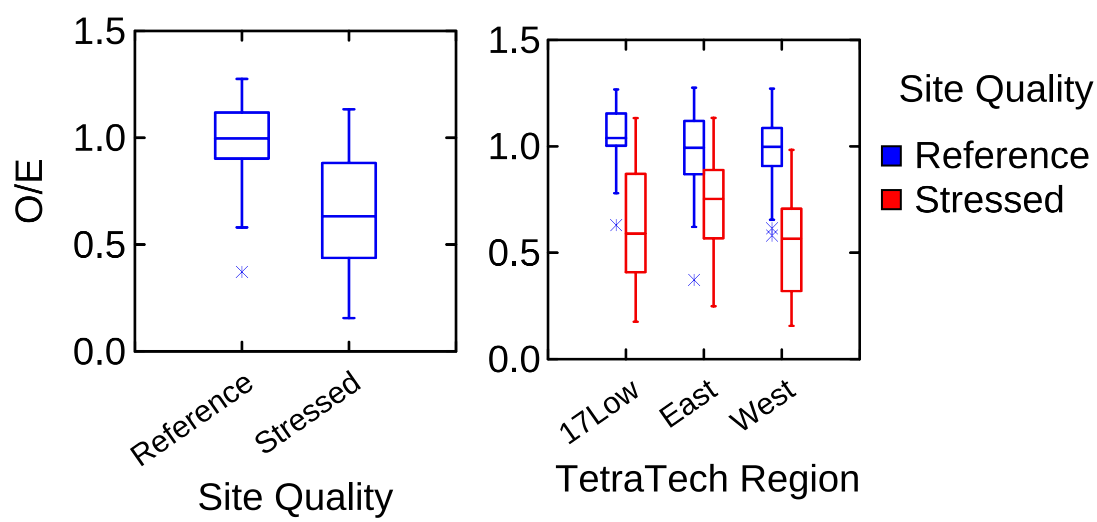

Figure 17. Boxplots showing discrimination between O/E values observed at reference sites and those at *a priori* selected stress sites. The left panel is for sites from all regions combined. The right panel separates sites based on the three regions used to construct and calibrate the multimetric indices.

5.3.2 Probability of Capture Matrix

The primary information used to estimate E is the predicted taxon-specific probabilities of capture that the model estimates. The web software provides these values for every taxon at every site submitted to the model. In general, this file will be of little use to the typical user but is provided for those users who are interested in scrutinizing model predictions.

5.3.3 Site O/E Values

O/E values for each of the samples submitted to the web software are provided in the O/E output file. This file lists estimates of O, E, and O/E for two different probability of capture thresholds: pc > 0 and pc > 0.5. Samples that were outside of the experience of the model are flagged (in red) in this file as well.

5.3.4 Taxa Sensitivity

An added feature of RIVPACS models is that the relative sensitivity of taxa to combined stressors occurring in a region can be assessed by determining the number of sites at which each taxon was observed and the number at which it was expected. The web model provides estimates of these sensitivities in the fourth output file. This file contains the number of assessed sites at which a taxon was observed, the number of sites it was expected to occur at, and the ratio of these two numbers. For the stressed sites examined while developing the MT model, several stoneflies and mayflies appeared to be especially sensitive to stress, whereas the cranefly *Tipula*, the caddis *Helicopsyche*, the mayfly *Callibaetis*, and Pyschodidae flies appeared to be tolerant (Appendix F).

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APPENDIX A

REFERENCE AND DEGRADED SITES

Table A-1. Reference sites used in development of biological indicators for Montana streams.

Station ID	Waterbody Name	Site Class	Latitude	Longitude
SHB-316	Albino	Mountains	47.5596	-113.537
J_S0009r	Arrow Creek	Plains	47.62564	-109.836
WMTP99-0716	Basin Creek	Mountains	46.67541	-110.44
YL_S0052	Beauvois Creek	Plains	45.44316	-108.163
M22BEVRC04	Beaver Creek	LowVal	47.0795	-109.599
EPA01-427	Bgsprng	Low Valley	47.0032	-109.344
EPA01-436	Big Ck	Mountains	45.3034	-110.94
SHB-328	Big Salmon	Mountains	47.529	-113.521
SHB-161	Bighorn	Mountains	47.1508	-112.643
C03BLACR01	Blackfoot R	Low Valley	46.90028	-113.755
WMTP99-R031	Blackleaf Creek	Mountains	48.01306	-112.693
M14BLKLC01	Blackleaf Creek	Plains	48.01278	-112.563
SHB-466	Blodgett	Mountains	46.276	-114.341
EPA01-431	Boulder R.	Mountains	45.323	-110.232
WMTP99-0623	Box Elder Creek	Plains	45.8448	-104.143
WMTP99-0803	Bracket Creek	Undetermined	45.85979	-110.855
WMTP99-0745	Browns Creek	Mountains	45.06537	-113.203
MAD-005	Cabin Creek	Mountains	44.8762	-111.34
SHB-496	Calf	Mountains	47.3385	-113.03
REFCAC	Calf Creek	Mountains	46.845	-110.96
WMTP99-R027	Calf Creek	Mountains	46.845	-110.96
EPA01-438	Cascade Ck	Mountains	45.3904	-111.24
YL_S0016	Cedar Cr.	Plains	46.79167	-104.558
EPA01-452	Chepat Ck	Mountains	48.7513	-114.727
REFCC	Clear Creek	Plains	48.30611	-109.491
WMTP99-0719	Clear Creek	Plains	48.27115	-109.526
YL_S0072up	Cow Creek	Plains	45.30903	-106.25
WMTP99-R032	Cow Creek	Plains	47.86111	-108.963
SHB-318	Cox	Mountains	48.069	-113.151
EPA01-429	Crookd	Mountains	45.1334	-108.428
WMTP99-0707	Crooked Creek	Undetermined	46.95293	-111.541
SHB-472	Dean	Mountains	47.906	-113.229
EPA01-449	Deerhorn Ck	Mountains	47.7129	-115.128
SHB-498	Dry Fork N.F. Blackfoot	Mountains	47.276	-113.002
WMTP99-0621	Dry Gulch Creek	Undetermined	46.55434	-111.134
EPA01-450	E. Fk. Bull R.	Mountains	48.1212	-115.698
SHB-182	E.F. Meadow	Mountains	47.1271	-112.8
M23EAGLC01	Eagle Creek	Low Valley	48.10083	-109.769
M23EAGLC03	Eagle Creek	Plains	47.91722	-110.053
REFEFBR	East Fork Bull River	Mountains	48.125	-115.728
M48RDWEC04	East Redwater Creek	Plains	47.758	-104.923
REFERC	East Rosebud Creek	Mountains	45.22667	-109.606
EPA01-440	Elk Ck	Low Valley	45.6267	-111.414
WMTP99-0628	Fish Creek	Plains	46.25091	-109.769
SHB-194	Flat	Mountains	46.5229	-113.62

Table A-1. Reference sites used in development of biological indicators for Montana streams.

Station ID	Waterbody Name	Site Class	Latitude	Longitude
C08FRSFK01	Flathead River - S Fork	Mountains	47.98423	-113.564
EPA01-432	Four Mile Ck	Mountains	45.3407	-110.246
WMTP99-0609	Fred Burr Creek	Undetermined	46.29743	-113.227
YNP-022	Gallatin R.	Mountains	44.9276	-111.047
EPA01-439	Gallitin R.	Mountains	45.3951	-111.207
SHB-474	Gorge	Mountains	47.76	-113.502
SHB-475	Graves	Mountains	47.737	-115.29
YNP-025	Grayling Cr.	Mountains	44.8853	-111.051
M04GHCNF01	Greenhorn Creek - North Fork	Mountains	45.12194	-112.039
WMTP99-0729	Highwood Creek	Low Valley	47.49904	-110.716
EPA01-443	Hot Springs Ck	Mountains	45.4529	-113.113
WMTP99-0607	Hungry Horse Creek	Undetermined	48.3536	-113.88
WMTP99-0715	Ingersol	Undetermined	45.35114	-109.56
WMTP99-0516	Keep Cool Creek	Undetermined	46.97382	-112.623
M03LMCHC01	Lamarche Creek	Mountains	45.91083	-113.217
YNP-060	Landslide Cr.	Low Valley	45.0376	-110.747
SHB-319	Lewis	Mountains	47.6596	-113.34
EPA01-428	Lfkrok	Mountains	45.0777	-109.424
SHB-455	Little Blackfoot	Mountains	46.422	-112.487
WMTP99-0633	Little Boulder River	Undetermined	46.167	-112.207
BKK070	Little Dry Creek	Plains	47.3413	-106.363
YL_S0006b	Little Missouri River	Plains	44.9952	-104.423
WMTP99-0648	Little Powder River	Plains	45.31896	-105.317
SHB-326	Little Salmon	Mountains	47.652	-113.369
WMTP99-0722	Lone Pine Creek	Mountains	47.21191	-112.495
SHB-220_C	Meadow	Mountains	47.122	-112.806
WMTP99-0515	Moose Creek	Mountains	48.82479	-114.521
WMTP99-0600	Moose Creek	Mountains	48.83676	-114.368
SHB-503	N.F. Fish	Mountains	46.928	-114.825
EPA01-423	Nfktet	Mountains	47.9711	-112.811
MAD-004	No Man Creek	Mountains	45.1144	-111.496
REFNFTR	North Fork Teton River	Mountains	47.96694	-112.808
WMTP99-R002	North Fork Teton River	Mountains	47.96694	-112.811
EPA01-441	O'Dell Creek	Low Valley	45.3408	-111.718
REFOFC2	Ofallon Creek	Plains	46.47111	-104.77
"106"	O'Fallon Creek	Plains	46.73498	-105.057
WMTP99-0604	O'Fallon Creek	Plains	46.47068	-104.77
M48PSTRC01	Pasture Creek	Plains	47.7064	-105.246
EPA01-435	Pine Ck	Mountains	45.5063	-110.789
WMTP99-0517	Pintler Creek	Mountains	45.90731	-113.48
"165"	Pumpkin Creek	Plains	46.18901	-105.622
SHB-239_A	Ranch	Mountains	46.524	-113.624
EPA01-448	Roaring Lion Creek	Mountains	46.1928	-114.257
REFRLC	Roaring Lion Creek	Mountains	46.19278	-114.243
SHB-505	Rock	Mountains	46.408	-112.967

Table A-1. Reference sites used in development of biological indicators for Montana streams.

Station ID	Waterbody Name	Site Class	Latitude	Longitude
"162"	Rock Creek	Plains	48.94098	-106.855
BKK124	Rock Creek	Plains	48.8783	-106.898
C02ROCKC01	Rock Creek	Low Valley	46.69583	-113.665
REFRC1	Rock Creek	Plains	48.87583	-106.897
REFRC2	Rock Creek	Plains	48.59028	-107.001
WMTP99-R005	Rock Creek	Plains	48.87583	-106.897
EPA01-453	S. Fk. Flathead R.	Mountains	47.8055	-113.414
EPA01-454	S. Fk. Willow Ck.	Mountains	45.6035	-111.896
SHB-315	Schafer	Mountains	48.064	-113.244
REFSEC	Seeley Creek	Mountains	45.09806	-109.299
EPA01-446	Seymore Ck.	Mountains	45.9985	-113.19
WMTP99-R015	Seymour Creek	Mountains	45.99583	-113.187
WMTP99-0507	Six Mile Creek	Low Valley	45.27109	-110.774
SHB-247	Sourdough	Mountains	47.152	-112.757
EPA01-424	South Fk. Sun River	Mountains	47.4916	-112.909
YNP-019	Specimen Cr.	Mountains	45.0127	-111.078
WMTP99-0549	Spring Creek	Plains	46.13696	-104.667
WMTP99-0838	Squaw Creek	Undetermined	47.08183	-111.594
YNP-104	Stephens Cr.	Low Valley	45.0371	-110.761
EPA01-447	Stony Ck	Mountains	46.2974	-113.67
SHB-362_B	Swan	Mountains	47.33	-113.768
EPA01-426	Tndrft	Low Valley	46.951	-111.164
LM_S0070EX	Tule Creek	Plains	48.18355	-105.491
SHB-324	Twentyfive	Mountains	48.157	-113.413
EPA01-162	Tygee Creek (Headwaters Of Henry'S Fk. R)	Mountains	44.6398	-111.256
WMTP99-0839	Unknown	Undetermined	45.54083	-111.899
EPA01-451	Vinal Ck	Mountains	48.8633	-115.619
SHB-506	W.F. Fish	Mountains	46.867	-114.818
EPA01-421	Waldrn	Mountains	47.9193	-112.817
WMTP99-R020	Waldron Creek	Mountains	47.92	-112.834
SHB-422	West Branch Big	Mountains	48.615	-115.474
MAD-003	West Fork Beaver Creek	Mountains	44.9053	-111.372
WMTP99-0705	West Fork Lolo Creek	Undetermined	46.68552	-114.558
REFWFPR	West Fork Poplar River	Plains	48.69694	-105.832
BKK162	Wf Stillwater	Mountains	45.3989	-109.96
EPA01-430	Wfkstl	Mountains	45.3988	-109.961
"167"	Whitewater Creek	Plains	48.60006	-107.519
LM_S0151up	Whitewater Creek	Plains	48.95661	-107.859
S03	Willow Creek	Plains	48.58472	-106.963
EPA01-442	Wisconsin Ck.	Mountains	45.5896	-113.334
REFWC	Wolf Creek At Wolf Point	Plains	48.08778	-105.678
LM_S0007ar	Woody Island Coulee	Plains	48.92265	-108.379
EPA01-434	Yellowstone R	Low Valley	45.5385	-110.581

Table A-2. Degraded sites used in development of biological indicators for Montana streams.

Station ID	Waterbody Name	Site Class	Latitude	Longitude
M41BEVRC02	Beaver Creek	Plains	48.2511111	-107.57222
"48"	Big Coulee Creek	Plains	46.180425	-109.25433
"92"	Big Muddy	Plains	48.720282	-104.484384
M50BMDYC01	Big Muddy Creek	Plains	48.21575	-104.688517
BKK017	Big Otter Creek	Plains	47.27	-110.73
BKK016	Big Otter Creek	Plains	47.2708	-110.7375
BKK015	Big Otter Creek	Plains	47.2658	-110.7067
BKK014	Big Otter Creek	Plains	47.2564	-110.68
BKK018	Big Otter Creek	Plains	47.3456	-110.8925
M20BSNDC01	Big Sandy Creek	Plains	48.45222	-109.91944
T02	Big Sandy Creek	Plains	48.45167	-109.91861
WMTP99-0611	BIGHORN	Plains	45.32087	-107.90763
C03BKBRC10	Black Bear Creek	Low Valleys	46.775833	-113.093611
"135"	Buffalo Creek	Plains	46.135849	-107.631996
BKK031	Butcher Creek	Plains	45.4194	-109.4331
BKK032	Butcher Creek	Plains	45.4831	-109.4525
M24CRLSC02	Careless Creek	Plains	46.371389	-109.281111
"161"	Charlie Creek	Plains	48.084995	-104.830693
CFRB-09	Clark Fork	Low Valleys	46.40085	-112.742283
WMTP99-0699	CURRANT CREEK	Plains	46.4108	-109.03573
C01DEEPC01	Deep Creek[1]	Unknown		
C03DOUGC20	Douglas Creek	Low Valleys	46.800833	-113.0644
C03DOUGC30	Douglas Creek	Low Valleys	46.861389	-113.0025
C03DOUGC10	Douglas Creek	Low Valleys	46.785278	-113.1275
"170"	East Forkrmells Creek	Plains	45.968107	-106.645416
BKK055	Flatwillow Creek	Plains	46.9331	-107.9383
M04GARDC01	Garden Creek	Mountains	45.245	-112.2167
M04GARDC02	Garden Creek	Low Valleys	45.224167	-112.1417
S05	Hanging Woman Cr	Plains	45.25444	-106.48999
YL_S0060Pr	Hanging Woman Creek	Plains	45.25444	-106.48999
M04IND01	Indian Creek	Mountains	45.499167	-112.131667
M04IND02	Indian Creek	Low Valleys	45.466389	-112.203333
C03JEFSC10	Jefferson Creek	Mountains	46.802222	-112.693611
C03JEFSC20	Jefferson Creek	Low Valleys	46.792222	-112.715
C03JEFSC30	Jefferson Creek	Low Valleys	46.776111	-112.738333
M08JEFFR01	Jefferson River	Low Valleys	45.894722	-111.598889
M01JONSC02	Jones Creek	Mountains	44.607278	-111.99525
M12LAKEC01	Lake Creek	Plains	47.714444	-111.560278
M41LARBC01	Larb Creek	Plains	48.1458333	-107.2916667
K01LIMEC01	Lime Creek	Mountains	48.660833	-114.889722
M51LMDYC01	Little Muddy	Plains	48.1302778	-104.1127778
WMTP99-0727	MCHESSOR CREEK	Mountains	45.3301	-112.30923
M01MTZLC01	Metzel Creek	Mountains	44.695556	-111.897222
WMTP99-0613	MIDDLE FORK BEAVER	Unknown	46.95863	-109.5488

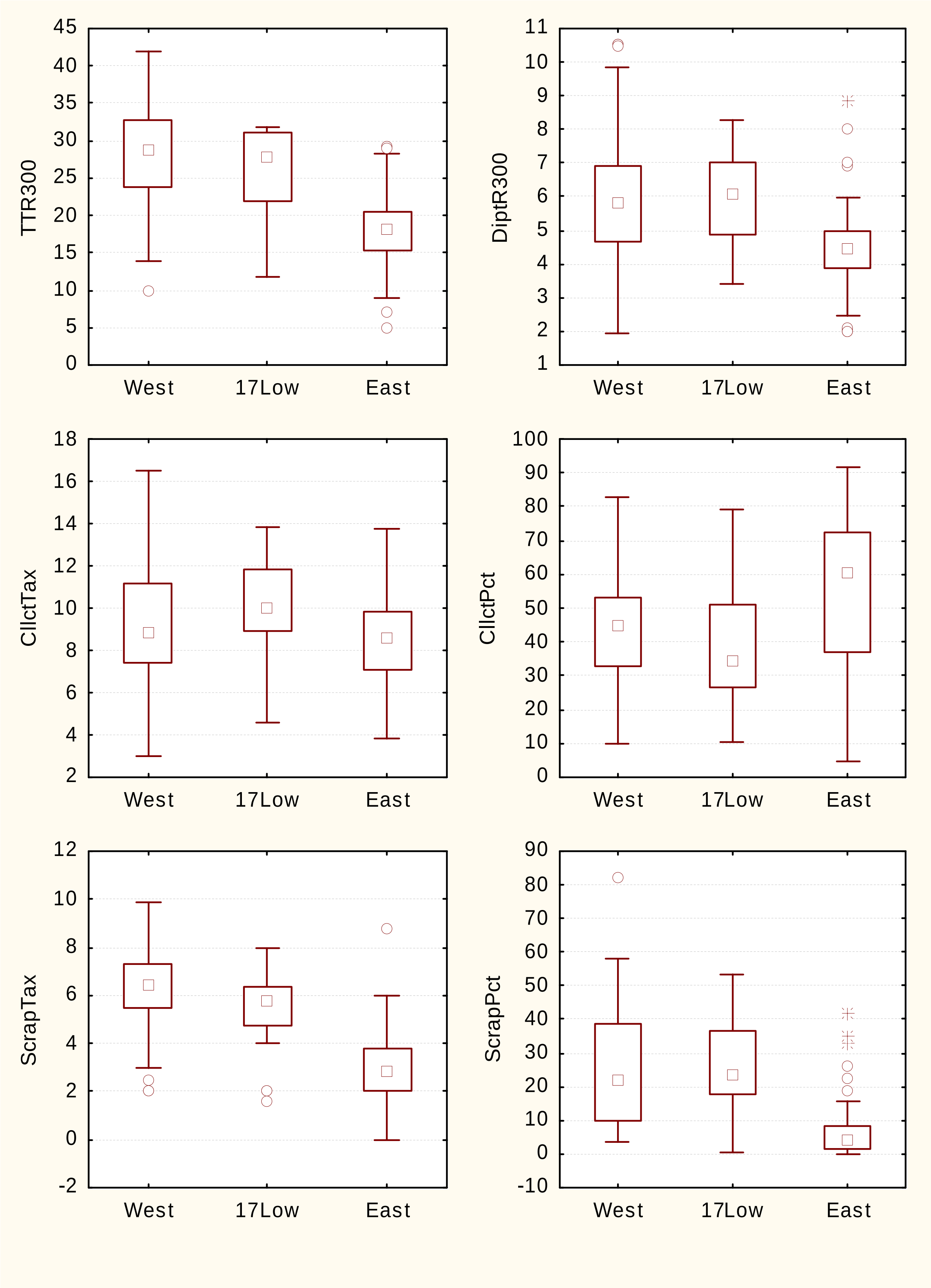
Table A-2. Degraded sites used in development of biological indicators for Montana streams.

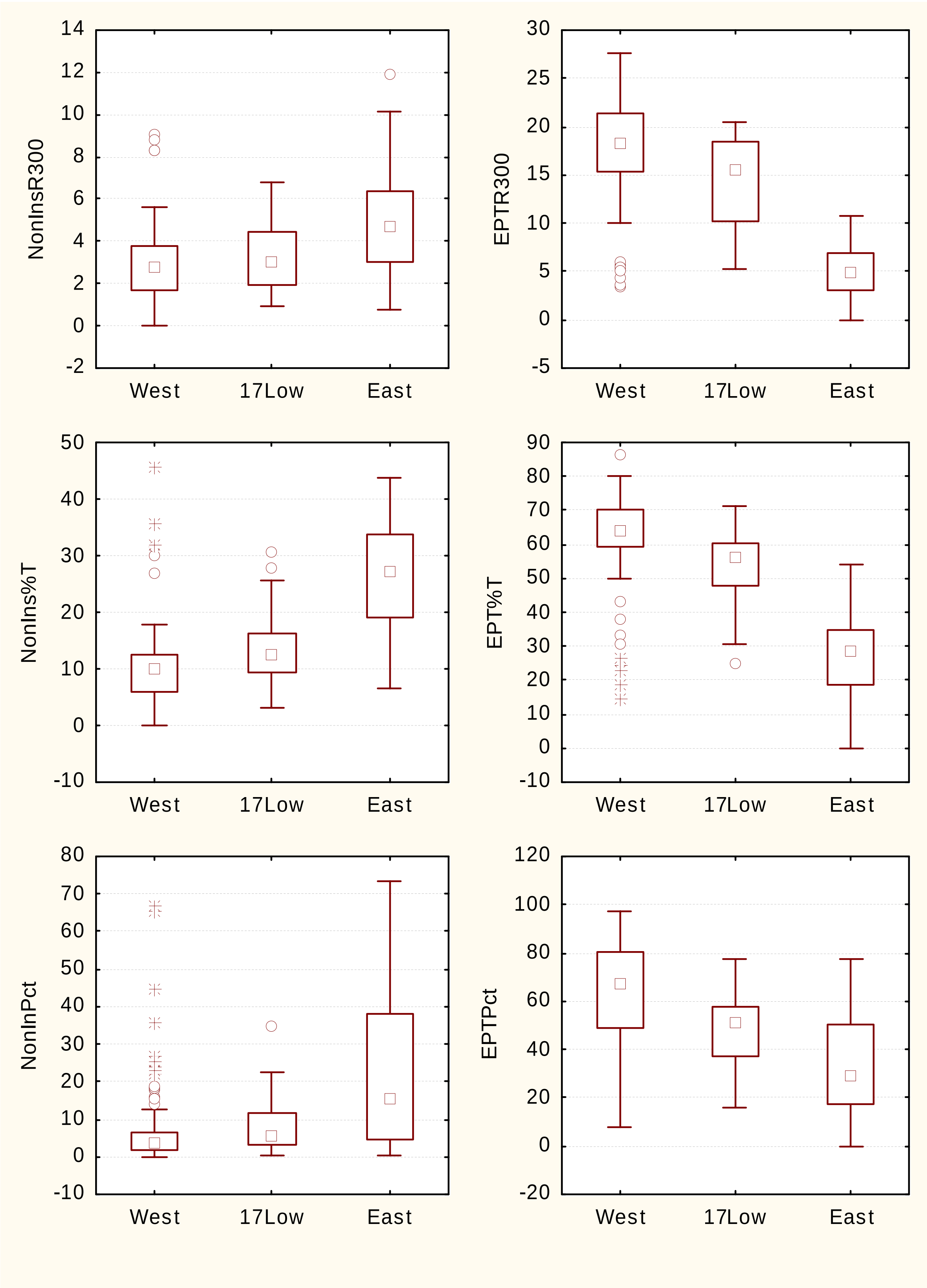
Station ID	Waterbody Name	Site Class	Latitude	Longitude
	CREEK[1]			
M45MILKR02	Milk River	Plains	48.507683	-107.21755
M04MORMC01	Mormon Creek	Mountains	45.190556	-112.172778
C03MURYC20	Murray Creek	Low Valleys	46.808611	-113.081944
C03MURYC10	Murray Creek	Low Valleys	46.808056	-113.136111
BKK088	Musselshell River	Low Valleys	46.4561	-110.195
M24MUSSR01	Musselshell River	Plains	46.428683	-109.843633
MU_S0004r	Musselshell River[1]	Unknown	46.45036	-110.1856
"200"	Otter Creek	Plains	45.512079	-106.173733
M01PEETC02	Peet Creek	Mountains	44.590833	-112.064167
BKK112	Pondera Coulee	Plains	48.1858	-111.3244
BKK113	Pondera Coulee	Plains	48.2692	-111.0661
M09PKPRC04	Prickly Pear Ck	Low Valleys	46.587528	-111.91927
M09PKPRC05	Prickly Pear Creek	Low Valleys	46.598028	-111.93055
BKK115	Red Rock River	Mountains	44.8449	-112.7719
BKK120	Red Rock River	Mountains	44.848	-112.7748
M48RDWR01	Redwater River	Plains	47.9280556	-105.2636111
T03	Sage Creek	Plains	48.52583	-110.28667
M03SWLGC01	Sawlog Creek	Mountains	45.837778	-113.25
M51SHGNC01	Shotgun Creek	Plains	48.1608333	-104.2466667
M50SMOKC01	Smoke Creek	Plains	48.3588889	-104.7461111
M03SWMPC02	Swamp Creek	Mountains	45.658889	-113.469722
M03SWMPC01	Swamp Creek	Mountains	45.629444	-113.5025
C03WALSC10	Wales Creek	Low Valleys	46.927778	-113.113333
C03WASHC10	Washington Creek	Low Valleys	46.785278	-112.665278
C03WASHC20	Washington Creek	Low Valleys	46.7625	-112.7
M45WIL0C01	Willow Creek South	Plains	48.1402778	-106.6266667
C03YRNMC20	Yourname Creek	Low Valleys	46.897778	-113.101389

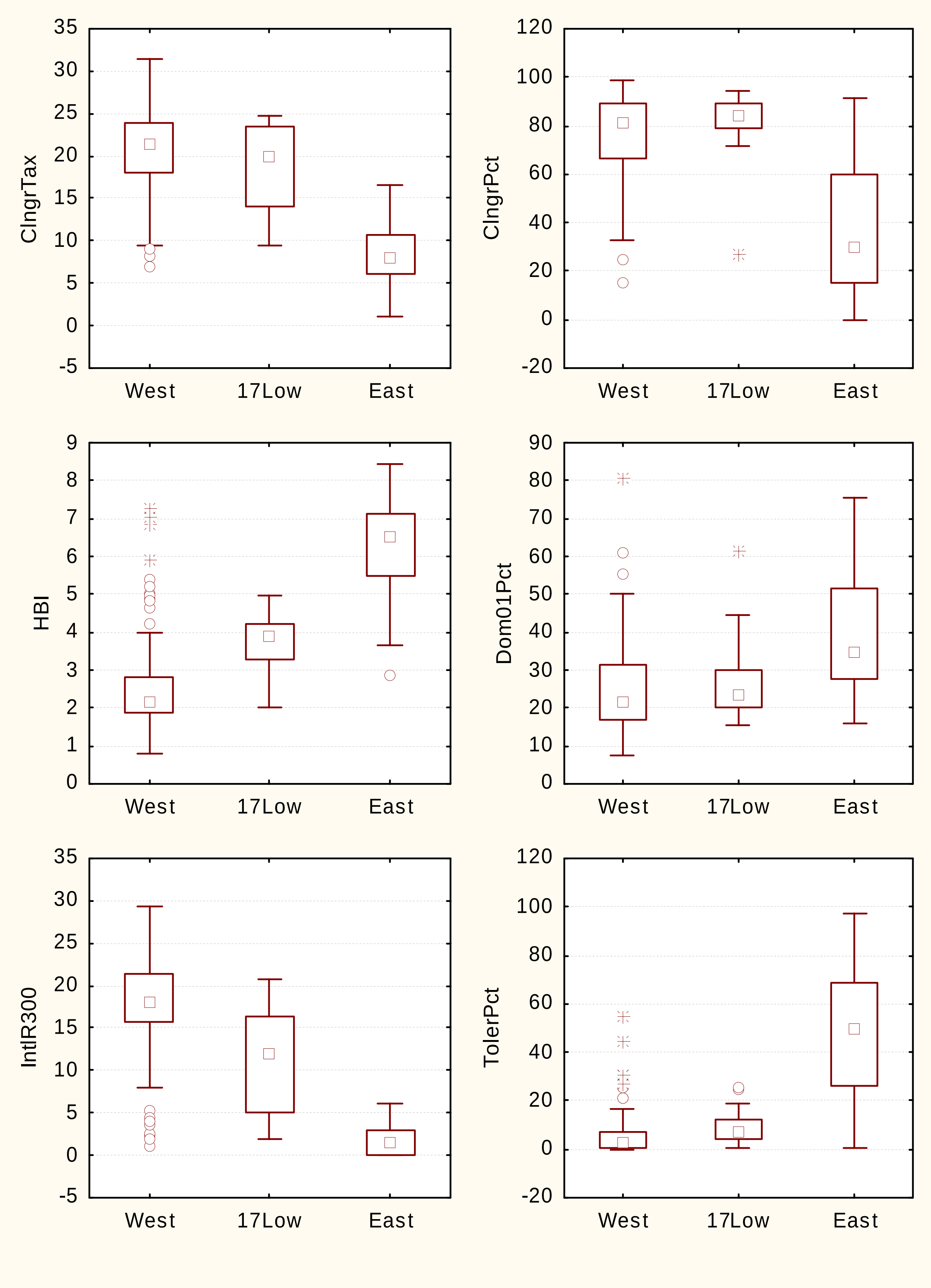
APPENDIX B

REFERENCE METRIC DISTRIBUTIONS BY SITE CLASS

The following figures show reference metric values in three site classes, the Mountains (West), Low Valleys (17Low), and Eastern Plains (East). Metric codes are as defined in Appendix B, Metric Statistics.


TTR300
DiptR300
CllctTax
CllctPct
ScrapTax
ScrapPct
West
17Low
East


NonInsR300
EPTR300
NonIns%T
EPT%T
NonInPct
EPTPct
West
17Low
East
14
12
10
8
6
4
2
0
-2
30
25
20
15
10
5
0
-5
50
40
30
20
10
0
-10
90
80
70
60
50
40
30
20
10
0
-10
80
70
60
50
40
30
20
10
0
-10
120
100
80
60
40
20
0
-20


ClngrTax
35
30
25
20
15
10
5
0
-5
West
17Low
East
ClngrPct
120
100
80
60
40
20
0
-20
West
17Low
East
HBI
9
8
7
6
5
4
3
2
1
0
West
17Low
East
Dom01Pct
90
80
70
60
50
40
30
20
10
0
West
17Low
East
IntlR300
35
30
25
20
15
10
5
0
-5
West
17Low
East
TolerPct
120
100
80
60
40
20
0
-20
West
17Low
East

APPENDIX C

METRIC STATISTICS

Metric discrimination efficiencies based on the 25th (DE25) and 75th (DE75) percentiles of reference values, including metric coefficients of variation (CV) for replicate samples.

Discrimination efficiencies in the Mountains and Plains reflect results of calibration data only. In the Plains, calibration samples were randomly selected twice, resulting in two different DEs. In the Low Valley site class, DEs were derived from all data.

CVs reflect sample variability based on duplicate samples collected using the traveling kick method from sites with neither reference nor degraded status.

Table Notes:
a – In the Plains, DEs are shown for two calibration sets – see text
b – Metrics are displayed in six metric categories and three site classes.
c – “R300” refers to rarefaction of all richness metrics to a target subsample of 300 organisms.
d – Genus level metrics (midges at genus) were calculated and evaluated where appropriate.
e – “na” – not applicable: DE incalculable due to invariable metric ranges.

Table C-1. Metric discrimination efficiencies based on the 25th (DE25) and 75th (DE75) percentiles of reference values, including metric coefficients of variation (CV) for replicate samples.

Metric Code	Metric Name	Mountains			Low Valleys			Plains[a]		
		DE25	DE75	CV	DE25	DE75	CV	DE25	DE75	CV
Richness[b]										
TTR300[c]	Total Taxa	9.1	18.2	12.0	23.5	23.5	12.2	54.2 / 41.7	12.5 / 25	12.9
TTR3GC	Total Taxa (midges at genus[d])	18.2	9.1	12.9	23.5	11.8	13.2	50 / 54.2	12.5 / 20.8	13.7
InsctR300	Insect Taxa	36.4	9.1	12.1	11.8	17.6	12.1	58.3 / 41.7	20.8 / 29.2	12.1
Insct%T	Insect Taxa Percent	81.8	0	4.1	11.8	47.1	4.7	33.3 / 37.5	16.7 / 16.7	3.5
NonInsR300	Non-Insect Taxa	0	72.7	55.7	58.8	23.5	29.8	37.5 / 25	16.7 / 12.5	35.2
NonIns%T	Non-Insect Taxa Percent	0	81.8	57.7	47.1	11.8	25.4	16.7 / 16.7	33.3 / 37.5	30.5
EPTR300	EPT Taxa	72.7	0	12.2	11.8	11.8	14.0	58.3 / 58.3	29.2 / 33.3	14.6
EPT%T	EPT Taxa Percent	100	0	9.9	47.1	29.4	10.1	50 / 58.3	33.3 / 41.7	14.3
EphmR300	Ephemeroptera Taxa	81.8	0	17.8	35.3	23.5	21.7	25 / 8.3	20.8 / 25	18.3
PlecR300	Plecoptera Taxa	81.8	0	27.9	11.8	17.6	41.3	0 / 0	16.7 / 16.7	58.0
TrchR300	Trichoptera Taxa	9.1	27.3	20.9	23.5	23.5	13.8	50 / 41.7	29.2 / 37.5	18.9
ColpR300	Coleoptera Taxa	0	90.9	38.6	0	47.1	40.7	62.5 / 41.7	16.7 / 16.7	41.0
DiptR300	Diptera Taxa	9.1	63.6	25.6	41.2	29.4	18.6	41.7 / 37.5	33.3 / 41.7	23.9
Dipt%T	Diptera Taxa Percent	9.1	63.6	28.8	52.9	11.8	15.8	41.7 / 20.8	41.7 / 33.3	17.7
DiptR3GC	Diptera Taxa (midges at genus)	0	27.3	25.6	47.1	17.6	18.5	62.5 / 41.7	16.7 / 20.8	25.7
ChirR300	Midge Taxa	0	63.6	20.0	41.2	5.9	25.2	37.5 / 33.3	25 / 12.5	32.1
ChirR3GC	Midge Taxa (midges at genus)	9.1	27.3	31.4	70.6	5.9	23.6	50 / 33.3	12.5 / 20.8	36.9
OrthR3GC	Orthocladiinae Taxa (midges at gen)	27.3	9.1	25.4	41.2	11.8	28.5	16.7 / 20.8	33.3 / 20.8	28.7
CrMuR300	Crustacea & Mollusca Taxa	0	90.9	471.4	5.9	58.8	31.4	33.3 / 29.2	8.3 / 20.8	59.7
CrMul%T	Crustacea & Mollusca Taxa Percent	0	90.9	468.4	0	41.2	24.7	41.7 / 29.2	33.3 / 20.8	53.9
OligR300	Oligochaeta Taxa	na[e]	na	45.9	na	na	59.1	na	na	42.4
Composition										
EPTPct	% EPT	81.8	0	21.3	52.9	23.5	14.7	41.7 / 41.7	37.5 / 33.3	12.7
EPTNoHB%	% EPT excluding Hydropsychidae and Baetidae	81.8	0	23.4	52.9	17.6	23.5	54.2 / 50	20.8 / 37.5	36.1
EphemPct	% Ephemeroptera	90.9	0	33.5	47.1	35.3	35.7	50 / 37.5	12.5 / 16.7	30.7
EphNoBaePct	% Ephemeroptera excluding Baetidae	81.8	0	41.1	29.4	35.3	34.2	62.5 / 33.3	12.5 / 25	54.3
PlecoPct	% Plecoptera	72.7	0	32.1	23.5	35.3	73.5	0 / 0	16.7 / 16.7	167.5

Metric Code	Metric Name	Mountains			Low Valleys			Plains[a]		
		DE25	DE75	CV	DE25	DE75	CV	DE25	DE75	CV
TrichPct	% Trichoptera	36.4	36.4	39.5	35.3	11.8	35.3	50 / 41.7	29.2 / 29.2	31.7
TriNoHydPct	% Trichoptera excluding Hydropsychidae	36.4	45.5	44.6	47.1	0	36.0	0 / 0	25 / 33.3	62.7
Baet2EphPct	% Baetidae:Ephemeroptera	36.4	36.4	52.3	64.7	17.6	13.6	0 / 20.8	37.5 / 58.3	21.3
Hyd2EPTPct	% Hydropsychidae:EPT	0	45.5	42.2	23.5	58.8	69.5	0 / 0	20.8 / 16.7	37.4
Hyd2TriPct	% Hydropsychidae:Trichoptera	0	18.2	48.0	23.5	47.1	28.1	0 / 0	12.5 / 20.8	28.9
DipPct	% Diptera	18.2	27.3	55.2	47.1	17.6	42.6	45.8 / 33.3	37.5 / 37.5	41.9
DipNoO%	% Diptera excluding Orthocladiinae	0	27.3	76.5	52.9	11.8	64.1	41.7 / 54.2	20.8 / 33.3	38.9
ChiroPct	% Midge	27.3	18.2	65.7	70.6	5.9	38.7	50 / 41.7	29.2 / 29.2	50.4
Chir2Dip%	% Midges:Diptera	54.5	18.2	27.0	88.2	0	23.5	41.7 / 29.2	33.3 / 16.7	22.0
TaChDi%	% Tanypodinae & Chironominae & Diamesinae	0	18.2	122.8	64.7	0	40.1	54.2 / 58.3	16.7 / 25	57.6
Tanypod%	% Tanypodinae	0	54.5	336.9	47.1	11.8	147.2	58.3 / 54.2	4.2 / 4.2	113.6
Diames%	% Diamesinae	0	27.3	211.7	0	11.8	46.2	0 / 0	16.7 / 20.8	114.2
Chirnae%	% Chironominae	9.1	18.2	110	64.7	0	56.7	37.5 / 50	16.7 / 20.8	80.2
Orthocl%	% Orthocladiinae	45.5	18.2	31.77	35.3	35.3	50.5	16.7 / 12.5	37.5 / 45.8	66.5
Orth2ChiPct	% Orthocladiinae:Midges	18.2	27.3	31.74	5.9	58.8	23.1	12.5 / 12.5	62.5 / 58.3	26.2
CrCh2Ch%	% Cricotopus&Chironomus:Midges	0	63.6	382.5	0	52.9	159.5	0 / 0	54.2 / 70.8	90.0
TanytPct	% Tanytarsini	0	36.4	117.9	58.8	0	151.5	33.3 / 41.7	25 / 33.3	83.5
Tnyt2ChiPct	% Tanytarsini:Midges	0	27.3	71.5	76.5	0	159.3	33.3 / 37.5	16.7 / 16.7	141.7
ColeoPct	% Coleoptera	0	63.6	39.5	5.9	41.2	34.9	62.5 / 50	12.5 / 29.2	70.7
OdonPct	% Odonata	0	36.4	0	0	17.6	475.8	0 / 54.2	25 / 33.3	154.0
NonInPct	% Non-Insect	0	90.9	67.1	41.2	23.5	48.0	41.7 / 12.5	25 / 29.2	47.0
AmphPct	% Amphipoda	0	27.3	632.5	0	23.5	217.4	0 / 41.7	16.7 / 16.7	48.1
BivalPct	% Bivalvia	0	81.8	632.5	0	47.1	125.7	0 / 0	25 / 25	235.2
CrMolPct	% Crustacea & Mollusca	0	90.9	331.9	5.9	64.7	79.9	20.8 / 16.7	16.7 / 16.7	52.2
GastrPct	% Gastropoda	0	72.7	468.9	0	41.2	96.2	50 / 45.8	33.3 / 12.5	96.9
IsoPct	% Isopoda	na	na	0	0	11.8	79.8	na / 0	na / 4.2	
OligoPct	% Oligochaeta	0	72.7	139.1	0	11.8	100.5	na / 20.8	na / 29.2	60.4
OligLee%	% Oligochaetes & Leaches	0	81.8	137.2	70.6	11.8	99.4	29.2 / 16.7	16.7 / 16.7	60.7
Shan_e	Shannon-Weiner Index (base e)	54.5	9.1	8.3	47.1	11.8	12.0	50 / 29.2	0 / 16.7	11.2

Metric Code	Metric Name	Mountains			Low Valleys			Plains[a]		
		DE25	DE75	CV	DE25	DE75	CV	DE25	DE75	CV
Evenness	Evenness	45.5	27.3	8.7	41.2	11.8	12.5	50 / 29.2	12.5 / 29.2	13.7
D_Mg	Margoleff's Diversity	54.5	9.1	12.5	29.4	23.5	12.9	50 / 41.7	12.5 / 25	13.8
D-Simp	Simpsons Diversity	9.1	54.5	36.6	17.6	41.2	37.8	0 / 12.5	50 / 25	39.2
Dom01Pct	% dominant 1	18.2	36.4	27.7	23.5	35.3	30.4	0 / 16.7	45.8 / 25	27.1
Dom2%	% dominant 2	9.1	54.5	26.4	17.6	35.3	18.8	4.2 / 20.8	41.7 / 25	15.6
Functional										
CllctPct	% Collector	18.2	45.5	25.6	29.4	35.3	24.7	33.3 / 37.5	54.2 / 37.5	28.3
FiltrPct	% Filterer	9.1	54.5	58.8	41.2	5.9	40.6	37.5 / 25	29.2 / 29.2	33.0
PredPct	% Predator	72.7	18.2	40.6	82.3	0	35.5	58.3 / 62.5	20.8 / 16.7	55.6
ScrapPct	% Scraper	45.5	27.3	23.8	23.5	58.8	28.8	41.7 / 33.3	29.2 / 33.3	43.9
PredScrap%	% Predator and Scraper	63.6	27.3	18.7	41.2	35.3	22.4	41.7 / 37.5	29.2 / 16.7	40.0
ShredPct	% Shredder	27.3	9.1	55.7	11.8	47.1	37.8	0 / 0	25 / 25	64.9
FiltColl%	% Collectors & Filterers	9.1	45.5	20.7	29.4	29.4	12.9	25 / 29.2	62.5 / 50	7.0
ScrpShrd%	% Scrapers & Shredders	54.5	36.4	22.7	17.6	52.9	22.3	41.7 / 33.3	29.2 / 33.3	40.3
CllctTax	Collector Taxa	0	63.6	11.2	41.2	29.4	18.3	45.8 / 41.7	20.8 / 16.7	20.9
Cllct%T	Collector Taxa Percent	0	81.8	9.3	52.9	17.6	11.7	29.2 / 29.2	45.8 / 33.3	13.2
FiltrTax	Filterer Taxa	9.1	63.6	20.0	29.4	23.5	15.0	20.8 / 8.3	29.2 / 33.3	18.6
Filt%T	Filterer Taxa Percent	9.1	63.6	25.5	29.4	0	17.4	12.5 / 12.5	16.7 / 41.7	18.2
PredTax	Predator Taxa	36.4	18.2	26.1	35.3	23.5	18.1	62.5 / 62.5	8.3 / 16.7	32.9
Pred%T	Predator Taxa Percent	45.5	18.2	24.1	35.3	17.6	14.7	41.7 / 54.2	33.3 / 29.2	30.2
ScrapTax	Scraper Taxa	63.6	9.1	17.1	23.5	47.1	18.9	50 / 0	20.8 / 29.2	27.2
Scrap%T	Scraper Taxa Percent	63.6	0	16.2	5.9	52.9	23.1	37.5 / 29.2	29.2 / 37.5	24.5
ShredTax	Shredder Taxa	9.1	0	40.3	11.8	64.7	45.3	0 / 0	16.7 / 20.8	58.7
Shred%T	Shredder Taxa Percent	27.3	0	35.2	11.8	58.8	39.8	0 / 0	25 / 20.8	67.5
PreShr%T	Predator and Shredder Taxa Percent	45.5	9.1	14.2	23.5	52.9	15.1	41.7 / 58.3	20.8 / 29.2	27.4
Habit										
BrrwrPct	% Burrower	0	90.9	30.8	35.3	41.2	26.0	45.8 / 41.7	33.3 / 25	42.6
ClmbrPct	% Climber	0	81.8	162.1	0	41.2	42.9	54.2 / 45.8	12.5 / 16.7	84.3
ClngrPct	% Clinger	72.7	0	7.5	29.4	17.6	8.2	41.7 / 29.2	33.3 / 33.3	9.1
SprwlPct	% Sprawler	27.3	27.3	42.7	52.9	11.8	21.7	33.3 / 29.2	25 / 16.7	39.3
SwmmrPct	% Swimmer	45.5	27.3	57.5	41.2	35.3	38.9	37.5 / 16.7	20.8 / 25	25.1

Metric Code	Metric Name	Mountains			Low Valleys			Plains[a]		
		DE25	DE75	CV	DE25	DE75	CV	DE25	DE75	CV
BrrwrTax	Burrower Taxa	0	90.9	26.5	23.5	52.9	25.5	37.5 / 25	8.3 / 4.2	27.8
Brrwr%T	Burrower Taxa Percent	0	100	22.5	11.8	41.2	18.7	25 / 29.2	29.2 / 29.2	22.4
ClmbrTax	Climber Taxa	0	81.8	98.1	0	23.5	54.6	45.8 / 45.8	4.2 / 12.5	52.7
Clmbr%T	Climber Taxa Percent	0	81.8	138.5	0	29.4	46.4	37.5 / 58.3	12.5 / 16.7	59.1
ClngrTax	Clinger Taxa	45.5	0	13.1	23.5	5.9	12.7	37.5 / 41.7	25 / 37.5	14.9
Clngr%T	Clinger Taxa Percent	81.8	0	7.6	29.4	23.5	6.3	37.5 / 41.7	33.3 / 37.5	10.7
SprwlTax	Sprawler Taxa	27.3	27.3	18.0	17.6	52.9	20.6	50 / 33.3	20.8 / 16.7	24.4
Sprwl%T	Sprawler Taxa Percent	18.2	18.2	18.6	23.5	47.1	16.2	37.5 / 29.2	45.8 / 33.3	21.7
SwmmrTax	Swimmer Taxa	18.2	54.5	31.9	29.4	47.1	24.0	37.5 / 37.5	25 / 12.5	21.9
Swmmr%T	Swimmer Taxa Percent	18.2	54.5	49.7	23.5	23.5	19.6	45.8 / 41.7	37.5 / 29.2	27.6
Voltinism										
MltVolPct	% Multivoltine	36.4	36.4	45.2	64.7	17.6	27.1	45.8 / 41.7	37.5 / 37.5	26.1
UniVolPct	% Univoltine	27.3	27.3	17.4	17.6	64.7	19.5	41.7 / 41.7	41.7 / 25	17.5
SemVolPct	% Semivoltine	0	18.2	0	0	11.8	475.8	0 / 0	29.2 / 25	104.9
SemVolTax	Semivoltine Taxa	0	18.2	0	0	11.8	244.9	0 / 0	29.2 / 25	83.8
UVolR300	Univoltine Taxa	54.5	9.1	14.2	35.3	41.2	14.73	58.3 / 45.8	8.3 / 16.7	16.7
Tolerance										
BeckR300	Beck's Index	81.8	0	16.4	29.4	23.5	16.0	45.8 / 41.7	29.2 / 33.3	19.8
BeckR3GC	Beck's Index (midges at genus)	81.8	0	16.3	23.5	23.5	16.7	45.8 / 41.7	29.2 / 29.2	17.4
HBI	Hilsenhoff's Index	0	90.9	25.0	17.6	41.2	11.7	33.3 / 33.3	37.5 / 37.5	5.6
HBI_GC	Hilsenhoff's Index (midges at genus)	0	90.9	16.9	11.8	41.2	10.6	29.2 / 33.3	45.8 / 45.8	5.4
IntolPct	% Intolerant	72.7	9.1	24.0	47.1	23.5	23.8	0 / 41.7	20.8 / 25	41.8
TolerPct	% Tolerant	0	72.7	98.5	64.7	23.5	48.1	45.8 / 45.8	25 / 16.7	37.2
IntlR300	Intolerant Taxa	81.8	9.1	18.2	17.6	11.8	16.3	0 / 41.7	29.2 / 29.2	22.1
Intol%T	Intolerant Taxa Percent	90.9	0	10.3	17.6	17.6	11.9	0 / 45.8	33.3 / 29.2	15.6
SensR300	MT Intolerant Taxa	90.9	0	28.0	0	17.6	128.1	0 / 0	16.7 / 8.3	84.6
TolrR300	Tolerant Taxa	0	90.9	45.6	23.5	29.4	28.4	50 / 54.2	20.8 / 16.7	31.0
Toler%T	Tolerant Taxa Percent	0	100	58.3	29.4	23.5	25.6	33.3 / 37.5	25 / 25	29.5
SupTol%	% Super-Tolerant	0	72.7	124.7	23.5	47.1	70.3	58.3 / 50	16.7 / 20.8	43.0

Appendix D

Index Alternatives

The following tables identify the metrics included in various index alternatives in each site class. The index title is at the top row of each column and metrics included in each index are noted with a similar label in the cells below the title. Statistics for evaluating the alternatives are in the lowest rows of each table.

All statistics were calculated for those indices that had promising discrimination efficiencies. Statistics are as follows:

Ref25th	25th percentile of the reference distribution of index values
DE25	Discrimination efficiency based on the Ref25th
MeanRef	Mean of index values in reference sites
MeanStress	Mean of index values in degraded sites
MeanDiff	Difference between MeanRef and MeanStress
StdDevRef	Standard deviation of reference site index values
MeanDiff/StdDevRef	MeanDiff divided by StdDevRef

In the Mountains, the index currently used by MT DEQ is labeled "MtnIBI".

In the Low Valleys, the index currently used by MT DEQ is labeled "FVPIBI". Index alternatives L1 through L16 show statistics for calibration data only. The FVPIBI and index alternatives AL17 through AL28 show statistics for all data.

In the Eastern Plains, the index currently used by MT DEQ is labeled "PIBI". Other indices that have been applied in the Plains of Montana and Wyoming include those developed by Marshall and Kerans (M&K), Bramblett and others for application with pool samples (BP), and the Wyoming Basin and Plains Index (WY). Scoring for metrics of these indices was based on the trend originally identified by the index authors (increasing or decreasing with stress), regardless of the trend exhibited in this data set.

Table D-1. Index alternatives tested in the Mountain site class.

Metric Name	MtnIBI	In_W1	In_W2	In_W3	In_W4	In_W5	In_W6	In_W7	In_W8
Total Taxa (rarefacted to 300)	MtnIBI								
Non-Insect Taxa Percent		1	2	3		5			
EPT Taxa (rarefacted to 300)	MtnIBI								
EPT Taxa Percent						5			
Ephemeroptera Taxa (rarefacted to 300)		1	2	3	4		6	7	8
Plecoptera Taxa (rarefacted to 300)		1	2	3	4		6	7	8
Coleoptera Taxa (rarefacted to 300)		1	2		4		6	7	8
Diptera Taxa Percent				3	4				
% EPT	MtnIBI	1		3		5		7	8
% Ephemeroptera			2		4		6		
% Plecoptera			2		4		6		
% Non-Insect		1		3	4		6	7	8
% Predator		1	2	3			6	7	
% Collectors & Filterers	MtnIBI				4	5			8
% Scrapers & Shredders	MtnIBI								
% Burrower		1	2	3	4	5			
Burrower Taxa Percent		1					6	7	8
Beck's Index		1	2						
Hilsenhoff's Index	MtnIBI			3	4	5	6	7	8
% dominant 1	MtnIBI								
Ref25th	67.7	67.5	62.2	63.5	60.2	71.1	65.6	67.6	69.5
DE25	66.7	**100**	**100**	88.9	88.9	88.9	**100**	**100**	88.9
MeanRef	73.5	71.6	65.9	69.5	67.2	77.6	69.3	73.1	74.3
MeanStress	56.6	37.1	34.1	38.6	34.4	48.1	33.0	37.2	39.4
MeanDiff	16.9	34.5	31.8	30.8	32.8	29.5	36.3	35.9	34.9
StdDevRef	12.7	10.6	10.9	10.5	12.3	13.0	12.4	11.8	12.4
MeanDiff/StdDevRef	1.3	3.3	2.9	2.9	2.7	2.3	2.9	3.0	2.8

Table D-1 (continued). Index alternatives tested in the Mountain site class.

Metric Name	In_W9	In_W10	In_W11	In_W12	In_W13	In_W14	In_W15	In_W16
EPT Taxa Percent					13	14	15	16
Ephemeroptera Taxa (rarefacted to 300)	9	10	11	12				
Plecoptera Taxa (rarefacted to 300)	9	10	11	12				
Coleoptera Taxa (rarefacted to 300)	9		11		13		15	
% EPT	9	10	11	12	13	14	15	16
% Non-Insect	9	10	11	12	13	14	15	16
% Predator	9	10	11		13	14		
% Collectors & Filterers				12			15	16
% Burrower			11					
Burrower Taxa Percent	9	10		12	13	14	15	16
Beck's Index	9							
Hilsenhoff's Index		10	11	12	13	14	15	16
Ref25th	66.0	66.7	67.6	67.0	74.4	62.2	74.2	72.8
DE25	**100.0**	**100.0**	88.9	88.9	**100.0**	**100.0**	**100.0**	88.9
MeanRef	70.4	71.8	72.4	73.1	78.1	66.4	79.4	78.9
MeanStress	34.8	35.6	38.0	38.1	41.4	34.4	43.9	43.1
MeanDiff	35.6	36.2	34.4	35.0	36.8	31.9	35.6	35.9
StdDevRef	11.8	12.5	10.7	13.1	12.1	10.8	12.9	13.5
MeanDiff/StdDevRef	3.0	2.9	3.2	2.7	3.0	3.0	2.7	2.6

Table D-2. Index alternatives tested in the Low Valley site class.

Metric Name	FVPIBI	Indx_L1	Indx_L2	Indx_L3	Indx_L4	Indx_L5	Indx_L6	Indx_L7	Indx_L8	Indx_L9
Total Taxa (rarefacted to 300)	FVPIBI									
EPT Taxa Percent		L1	L2	L3						L9
Ephemeroptera Taxa (rarefacted to 300)	FVPIBI									
Plecoptera Taxa (rarefacted to 300)	FVPIBI									
Trichoptera Taxa (rarefacted to 300)	FVPIBI				L4	L5		L7	L8	
Midge Taxa (midges at genus, rarefacted to 300)							L6		L8	
Crustacea & Mollusca Taxa (rarefacted to 300)							L6			
% Midges:Diptera						L5				
% Tanypodinae&Chironominae&Diamesinae		L1	L2	L3						
% Chironominae					L4			L7	L8	L9
% Orthocladiinae:Midges		L1	L2	L3						
% Tanytarsini							L6			
% Collector		L1		L3		L5				
% Filterer	FVPIBI									
Predator Taxa Percent					L4		L6	L7	L8	L9
Shredder Taxa (rarefacted to 300)										
Shredder Taxa Percent		L1	L2		L4		L6	L7	L8	L9
Burrower Taxa Percent		L1	L2	L3		L5				
Climber Taxa Percent		L1								
Clinger Taxa Percent					L4	L5	L6	L7	L8	L9
% Super-Tolerant					L4					
% Intolerant	FVPIBI									
% Tolerant	FVPIBI									
Ref 25th %ile	53.5	51.7	48.7	44.2	62.2	67.9	58.9	60	59.8	62.6
DE	47.4	73.3	73.3	66. 7	73.3	86. 7	80	86. 7	86. 7	73.3

Table D-2 (continued). Index alternatives tested in the Low Valley site class.

Metric Name	In_L10	In_L11	In_L12	In_L13	In_L14	In_L15	In_L16	In_AL17	In_AL18	In_AL19
Non-Insect Taxa (rarefacted to 300)								AL17		
EPT Taxa Percent								AL17	AL18	
Ephemeroptera Taxa (rarefacted to 300)			L12	L13						
Plecoptera Taxa (rarefacted to 300)										
Trichoptera Taxa (rarefacted to 300)	L10	L11	L12	L13	L14	L15	L16			
Crustacea & Mollusca Taxa (rarefacted to 300)					L14				AL18	
% EPT excluding Hydropsychids and Baetids								AL17	AL18	AL19
% Midge		L11								
% Midges:Diptera						L15	L16	AL17	AL18	AL19
% Chironominae	L10		L12	L13	L14					
% Crustacea & Mollusca									AL18	AL19
Predator Taxa Percent				L13			L16			
Shredder Taxa (rarefacted to 300)								AL17	AL18	AL19
Shredder Taxa Percent				L13			L16			
Predator & Shredder Taxa Percent	L10	L11	L12		L14	L15				
Burrower Taxa (rarefacted to 300)								AL17	AL18	
Clinger Taxa Percent	L10	L11	L12	L13	L14	L15	L16			
% Intolerant								AL17	AL18	AL19
Ref 25th %ile	55.7	57.3	52.9	55.7	50.6	70.4	72.5			60.8
DE	86. 7	73.3	73.3	73.3	66. 7	93.3	93.3	68.4	68.4	78.9
MeanRef								61. 2	66.8	65.2
MeanStressed								48.1	52.0	47.0
MeanDiff								13.1	14.8	18.2
StdDevRef								10.1	12.8	13.6
								1.3	1.2	1.3

Table D-2 (continued). Index alternatives tested in the Low Valley site class.

Metric Name	In_AL20	In_AL21	In_AL22	In_AL23	In_AL24	In_AL25	In_AL26	In_AL27	In_AL28
EPT Taxa Percent					AL24	AL25			AL28
Midge Taxa (midges at genus, rarefacted to 300)					AL24				
% EPT excluding Hydropsychids and Baetids				AL23	AL24	AL25	AL26	AL27	
% Midge			AL22	AL23		AL25	AL26	AL27	AL28
% Midges:Diptera	AL20	AL21			AL24				
% Crustacea & Mollusca	AL20	AL21	AL22		AL24		AL26	AL27	AL28
Shredder Taxa (rarefacted to 300)	AL20		AL22		AL24			AL27	
Shredder Taxa Percent		AL21		AL23		AL25	AL26		AL28
% Intolerant	AL20	AL21	AL22	AL23	AL24	AL25	AL26	AL27	AL28
Ref 25th %ile	63.2	70.1	55.7	58.2	60.0	54.0	58.2	56.0	62.2
DE	84.2	**89.5**	78.9	**89.5**	78.9	84.2	**89.5**	78.9	78.9
MeanRef	70.6	72.5	64.5	61.8	66.4	58.7	61.8		
MeanStressed	49.8	55.0	43.8	46.4	49.3	46.3	46.4		
MeanDiff	20.8	17.5	20.7	15.4	17.1	12.3	15.4		
StdDevRef	12.8	12.2	12.1	12.0	13.4	11.3	12.0		
	1.6	1.4	1.7	1.3	1.3	1.1	1.3		

Table D-3. Index alternatives tested in the Eastern Plains site class.

Metric Name	PrairieIBI	M&K	BramblettPool	WY
Total Taxa (rarefacted to 300)				WY
Non-Insect Taxa (rarefacted to 300)		M&K		
EPT Taxa (rarefacted to 300)	PIBI			
Ephemeroptera Taxa (rarefacted to 300)			BP	WY
Plecoptera Taxa (rarefacted to 300)				WY
Trichoptera Taxa (rarefacted to 300)			BP	WY
% EPT	PIBI		BP	
% Plecoptera				WY
% Trichoptera excluding Hydropsychidae				WY
% Diptera		M&K		
% Midges:Diptera		M&K		
% Non-Insect			BP	WY
% Oligochaetes & Leaches	PIBI			
% Predator		M&K		
% Scraper				WY
Filterer Taxa (rarefacted to 300)	PIBI		BP	
% Clinger	PIBI			
% Swimmer		M&K		
Swimmer Taxa (rarefacted to 300)	PIBI			
Semivoltine Taxa (rarefacted to 300)				WY
Univoltine Taxa (rarefacted to 300)	PIBI		BP	
Hilsenhoff's Index		M&K		
% Super-Tolerant			BP	
% dominant 2	PIBI			
% Intolerant	PIBI			
Ref25th	38.0	47.1	32.9	22.1
DE25	37.5	20.8	37.5	58.3

Table D-3 (continued). Index alternatives tested in the Eastern Plains site class.

Metric Name	In_E1	In_E2	In_E3	In_E4	In_E5	In_E6	In_E7	In_E8	In_E9
Total Taxa (rarefacted to 300)			E3						
Insect Taxa (rarefacted to 300)	E1	E2			E5	E6		E8	
EPT Taxa (rarefacted to 300)				E4					
Coleoptera Taxa (rarefacted to 300)			E3	E4			E7		
Diptera Taxa (midges at genus, rarefacted to 300)							E7		E9
Shannon-Weiner Index (base e)				E4		E6	E7	E8	E9
% Ephemeroptera					E5				
% Trichoptera	E1	E2	E3	E4					
% Tanypodinae&Chironominae&Diamesinae	E1	E2	E3	E4					
% Tanypodinae									
% Orthocladiinae:Midges	E1	E2	E3	E4	E5	E6		E8	E9
% Coleoptera	E1	E2		E4					
% Predator			E3			E6			E9
% Scraper			E3		E5	E6	E7	E8	E9
Scraper Taxa (rarefacted to 300)	E1	E2		E4					
Univoltine Taxa (rarefacted to 300)					E5	E6	E7	E8	E9
% dominant 1	E1								
% dominant 2					E5				
Ref25th	39.4	33.9	39.2	38.8	44.8	46.7	47.9	48.5	48.4
DE25	58.3	54.2	45.8	62.5	66.7	**70.8**	66.7	66.7	**70.8**

Table D-3 (continued). Index alternatives tested in the Eastern Plains site class.

Metric Name	In_E10	In_E11	In_E12	In_E13	In_E14	In_E15	In_E16	In_E17	In_E18
Insect Taxa (rarefacted to 300)	E10	E11	E12	E13	E14	E15	E16	E17	E18
Diptera Taxa (midges at genus, rarefacted to 300)	E10								
Shannon-Weiner Index (base e)	E10	E11	E12	E13		E15	E16	E17	E18
EphNoBaePct			E12						
% Chironominae				E13	E14	E15	E16	E17	E18
% Orthocladiinae:Midges	E10	E11	E12				E16		
% Predator	E10	E11	E12	E13	E14				
% Scraper	E10	E11	E12	E13	E14	E15	E16		E18
PredScrap%								E17	
Clinger Taxa (rarefacted to 300)		E11							
Univoltine Taxa (rarefacted to 300)	E10	E11	E12	E13	E14	E15	E16	E17	
Ref25th	48.2	45.8	44.1	39.1	34.3	40.9	44.9	44.3	39.5
DE25	**70.8**	62.5	66.7	**70.8**	66.7	**70.8**	66.7	62.5	66.7

Table D-3 (continued). Index alternatives tested in the Eastern Plains site class.

Metric Name	In_E19	In_E20	In_E21	In_E22	In_E23	In_E24	In_E25	In_E26
Insect Taxa (rarefacted to 300)		E20		E22			E25	E26
Coleoptera Taxa (rarefacted to 300)		E20	E21					
Diptera Taxa (midges at genus, rarefacted to 300)				E22	E23			
Shannon-Weiner Index (base e)	E19	E20	E21	E22	E23	E24	E25	E26
% Trichoptera						E24		
% Ephemeroptera excluding Baetidae						E24		
% Chironominae	E19	E20	E21	E22	E23	E24	E25	E26
% Coleoptera							E25	E26
% Predator								E26
% Scraper	E19	E20	E21	E22	E23	E24		E26
% Predator & Scraper							E25	
Univoltine Taxa (rarefacted to 300)	E19		E21		E23	E24		
Ref25th	38.6	48.3	48.2	50.7	53.1	33.9	38.7	34.3
DE25	66.7	62.5	58.3	58.3	62.5	58.3	66.7	66.7

APPENDIX E

OTU MAPPING TABLE

This appendix is an MSExcel file created by C. Hawkins, and is titled "MT-Master_Taxa_File_26Sept2005". It contains all taxa names in the database used for these indicators analyses, and maps the decisions used to arrive at the final OTU. The spreadsheet file is over 2800 rows long and is excessive for inclusion as a hardcopy appendix to this report. The file can be obtained by directly contacting any of the authors, Montana DEQ (D. Feldman [406-444-6764]), USEPA (T. Laidlaw [406-457-5016]), or via the following URLs:

http://n-steps.tetratech-ffx.com/reports/MT-Master_Taxa_File_26Sept2005.xls

www.cnr.usu.edu/wmc (Predictive Models/Montana Data)

APPENDIX F

TAXA SENSITIVITY

Appendix F. Summary of observed and expected taxon frequencies of occurrence at stressed sites. The sensitivity index (SI) is calculated as (sites observed / sites expected). Interpretation of SI values must be tempered by consideration of both the observed and expected number of sites.

Taxon	Mean Probability of Capture	Number Sites Expected	Number Sites Observed	Sensitivity Index
Megarcys	0.137408	9.343768	0	0
Yoraperla	0.128995	8.771692	0	0
Glutops	0.093127	6.332643	0	0
Rhyacophila_hyalinata_group	0.086212	5.862393	0	0
Doroneuria	0.082122	5.584282	0	0
Rhyacophila_betteni_group	0.071141	4.837607	0	0
Dixa	0.057642	3.919654	0	0
Paraperla	0.054908	3.733734	0	0
Neothremma	0.054812	3.727206	0	0
Atherix	0.051105	3.475157	0	0
Kogotus	0.049463	3.363481	0	0
Rhyacophila_angelita_group	0.044829	3.048403	0	0
Argia	0.041754	2.839283	0	0
Dicosmoecus	0.041025	2.78972	0	0
Blephariceridae	0.035037	2.382499	0	0
Skwala	0.03304	2.246708	0	0
Erpobdellidae	0.03218	2.188219	0	0
Visoka	0.030687	2.086695	0	0
Other_Hydrobiidae	0.029142	1.981652	0	0
Berosus	0.028723	1.953193	0	0
Rhyacophila_vofixa_group	0.02869	1.950903	0	0
Rhyacophila_coloradensis_group	0.0285	1.938002	0	0
Oreogeton	0.027406	1.863603	0	0
Rhyacophila_verrula_group	0.025505	1.734339	0	0
Rhyacophila_sibirica_group	0.024863	1.69069	0	0
Lepidoptera	0.022605	1.537155	0	0
Hydraena	0.022605	1.537155	0	0
Maruina	0.022605	1.537155	0	0
Forcipomyiinae	0.022605	1.537155	0	0
Calineuria	0.022605	1.537155	0	0
Podmosta	0.022605	1.537155	0	0
Gammarus	0.022605	1.537155	0	0
Apatania	0.019514	1.326964	0	0
Ecclisomyia	0.018968	1.289842	0	0
Cryptochia	0.017613	1.1977	0	0
Deuterophlebia	0.014974	1.018258	0	0
Kathroperla	0.013619	0.926117	0	0

Wiedemannia	0.011077	0.753203	0	0
Chaoborus	0.009574	0.651064	0	0
Anax	0.009574	0.651064	0	0
Aeshna	0.009574	0.651064	0	0
Phryganeidae	0.009574	0.651064	0	0
Cambaridae	0.009574	0.651064	0	0
Psychoda	0.009574	0.651064	0	0
Neochoroterpes	0.009574	0.651064	0	0
Ochthebius	0.009574	0.651064	0	0
Sciomyzidae	0.009574	0.651064	0	0
Cinygma	0.006537	0.444497	0	0
Pictetiella	0.006537	0.444497	0	0
Acerpenna	0.005895	0.400847	0	0
Tanyderidae	0.005895	0.400847	0	0
Leuctridae	0.190915	12.982203	1	0.077029
Parapsyche	0.189987	12.919126	1	0.077405
Clinocera	0.129782	8.825182	1	0.113312
Drunella_coloradensis_flavilinea	0.243611	16.565559	2	0.120732
Claassenia_sabulosa	0.118921	8.086621	1	0.123661
Drunella_doddsi	0.375008	25.500578	4	0.156859
Acarina	0.615856	41.878185	7	0.167151
Rhithrogena	0.34292	23.318584	4	0.171537
Other_Chloroperlidae	0.511186	34.760677	6	0.172609
Pteronarcys	0.067816	4.611464	1	0.216851
Ameletus	0.257779	17.528954	4	0.228194
Epeorus	0.377576	25.675177	6	0.233689
Psychoglypha	0.062895	4.276852	1	0.233817
Limnephilus	0.054785	3.725374	1	0.268429
Serratella	0.158732	10.793778	3	0.277938
Pedicia	0.051105	3.475157	1	0.287757
Cinygmula	0.432936	29.439659	10	0.339678
Drunella_spinifera	0.165008	11.220532	4	0.356489
Caudatella	0.040551	2.75744	1	0.362655
Hexatoma	0.273374	18.589436	7	0.376558
Glossosoma	0.306911	20.869955	8	0.383326
Pisidiidae	0.268509	18.258602	7	0.383381
Other_Ephemerella	0.149008	10.13257	4	0.394767
Dolophilodes	0.066769	4.540272	2	0.440502
Acentrella	0.194341	13.21519	6	0.454023
Ephydridae	0.03218	2.188219	1	0.456993
Ordobrevia	0.03218	2.188219	1	0.456993
Diphetor	0.231609	15.749392	8	0.507956
Arctopsyche	0.18733	12.738413	7	0.549519
Neophylax	0.026051	1.771461	1	0.564506
Taeniopterygidae	0.051556	3.505801	2	0.570483

Hesperoperla	0.150011	10.200743	6	0.588192
Nematoda	0.364338	24.774965	15	0.60545
Capniidae	0.096051	6.531463	4	0.61242
Rhabdomastix	0.069169	4.703501	3	0.637823
Cleptelmis	0.114261	7.769752	5	0.643521
Agapetus	0.022605	1.537155	1	0.650553
Plauditus	0.022605	1.537155	1	0.650553
Baetis	0.812685	55.262585	37	0.669531
Micrasema	0.236223	16.063137	11	0.684798
Zapada	0.39894	27.127935	19	0.700385
Dubiraphia	0.230582	15.679557	11	0.70155
Dicranota	0.288823	19.639982	14	0.712832
Sialis	0.102657	6.980695	5	0.716261
Hydroptila	0.199991	13.599366	10	0.735328
Zaitzevia	0.079605	5.413159	4	0.73894
Glossiphoniidae	0.07739	5.262528	4	0.760091
Hexagenia	0.019149	1.302129	1	0.767973
Turbellaria	0.228543	15.540929	12	0.772155
Anagapetus	0.018968	1.289842	1	0.775289
Paraleptophlebia	0.242912	16.517982	13	0.787021
Chironominae	0.939815	63.907433	52	0.813677
Oligophlebodes	0.017613	1.1977	1	0.834934
Antocha	0.158116	10.751919	9	0.83706
Naucoridae	0.051329	3.490348	3	0.859513
Agabus	0.051329	3.490348	3	0.859513
Prodiamesinae	0.067816	4.611464	4	0.867404
Chelifera_Metachela_Neoplasta	0.168717	11.472737	10	0.871632
Diamesinae	0.364604	24.793076	22	0.887345
Heterlimnius	0.310571	21.118837	19	0.899671
Orthocladiinae	0.916492	62.321434	57	0.914613
Polycentropus	0.015469	1.051912	1	0.95065
Ormosia	0.015469	1.051912	1	0.95065
Hydropsyche_Ceratopsyche	0.408315	27.765402	27	0.972433
Hemerodromia	0.04521	3.074309	3	0.975829
Other_Oligochaeta	0.610969	41.545872	41	0.986861
Tanypodinae	0.558403	37.971432	38	1.000752
Optioservus	0.4674	31.783218	32	1.006821
Ceratopogoninae	0.307251	20.893087	22	1.05298
Simuliidae	0.578297	39.324211	42	1.068044
Caenis	0.217551	14.793467	16	1.081558
Brachycentrus	0.314399	21.379114	24	1.122591
Hyalella	0.233815	15.899392	18	1.132119
Tricorythodes	0.145206	9.873993	12	1.215314
Haliplus	0.070478	4.792476	6	1.251962
Planorbidae	0.070478	4.792476	6	1.251962

Ochrotrichia	0.022605	1.537155	2	1.301105
Corixidae	0.185371	12.605247	17	1.348645
Gomphidae	0.086965	5.913593	8	1.352816
Nectopsyche	0.03218	2.188219	3	1.370978
Lepidostoma	0.202971	13.802008	19	1.376611
Physa_Physella	0.334828	22.76828	32	1.405464
Other_Coenagrionidae	0.156648	10.652055	15	1.408179
Other_Lymnaeidae	0.083285	5.663376	8	1.412585
Cheumatopsyche	0.238289	16.203623	23	1.419436
Fallceon	0.038298	2.604257	4	1.535947
Culicidae	0.009574	0.651064	1	1.535947
Peltodytes	0.009574	0.651064	1	1.535947
Rhantus	0.009574	0.651064	1	1.535947
Tropisternus	0.009574	0.651064	1	1.535947
Stenonema	0.009574	0.651064	1	1.535947
Rhyacophila_brunnea_vemna_groups	0.156998	10.675884	17	1.592374
Oecetis	0.099995	6.799683	12	1.764788
Isoperla	0.041573	2.826997	5	1.768661
Leptophlebia	0.022605	1.537155	3	1.951658
Brychius	0.022605	1.537155	3	1.951658
Laccobius	0.022605	1.537155	3	1.951658
Oreodytes	0.022605	1.537155	3	1.951658
Lara	0.063537	4.320502	9	2.083091
Malenka	0.074352	5.055962	11	2.17565
Rhyacophilla_vagrita_group	0.006537	0.444497	1	2.249732
Podonominae	0.006537	0.444497	1	2.249732
Notonectidae	0.019149	1.302129	3	2.30392
Narpus	0.079224	5.387252	13	2.413104
Rhyacophila_alberta_group	0.005895	0.400847	1	2.494714
Tipula	0.122861	8.354581	21	2.513591
Helicopsyche	0.086965	5.913593	15	2.536529
Drunella_grandis	0.04029	2.739697	7	2.555027
Hesperophylax	0.022605	1.537155	4	2.602211
Callibaetis	0.067021	4.55745	12	2.633051
Pericoma_Telmatoscopus	0.065082	4.425544	12	2.711531
Libellulidae	0.019149	1.302129	4	3.071893
Laccophilus	0.009574	0.651064	2	3.071893
Pteronarcella	0.022605	1.537155	5	3.252763
Muscidae	0.029142	1.981652	9	4.541665
Heptagenia	0.009574	0.651064	3	4.60784
Ptychopteridae	0.01179	0.801695	4	4.989429
Agraylea	0.01179	0.801695	4	4.989429
Stenelmis	0.019149	1.302129	7	5.375813
Tabanidae	0.019149	1.302129	8	6.143786
Helophorus	0.009574	0.651064	4	6.143786

Setvena	0.011077	0.753203	5	6.638318
Amiocentrus	0.01179	0.801695	6	7.484144
Limnophila	0.006537	0.444497	4	8.99893
Hesperoconopa	0.00454	0.308706	4	12.957333
Hedriodiscus_Odontomyia	0	0	1	999
Laccornis	0	0	1	999
Amphinemura	0	0	2	999
Ephemerella_aurivillii	0	0	5	999
Microcylloepus	0	0	2	999
Isogenoides	0	0	2	999
Helichus	0	0	1	999
Attenella	0	0	2	999
Choroterpes	0	0	1	999
Asellidae	0	0	2	999
Liodessus	0	0	2	999
Hydroporus	0	0	1	999
Nematomorpha	0	0	1	999
Ceraclea	0	0	2	999
Siphlonuridae	0	0	1	999
Timpanoga_hecuba	0	0	1	999
Caloparyphus_Euparyphus	0	0	1	999
Hygrotus	0	0	1	999